Chemical synthesis of small molecule libraries around the p-benzoquinone scaffold

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1 Introduction

Paul Ehrlich discovered that methylene blue could stain nerve cells and he promoted the idea that low molecular-weight organic compounds could be of value for studying receptors in biological systems.[1,2] The use of such small molecules instead of genetic mutations to establish a link between gene-products and their functions is called chemical genetics. Chemical genetics relies on a collection of compounds that are able to change the way proteins work directly in real time rather then indirectly by manipulating their genes.

Natural products are known to bind to proteins and may therefore serve as a fruitful source of inspiration for the synthesis of compound collections. The natural product quinone family contains 1,4-benzoquinone core **1** (Figure *1*) and is documented to affect a wide variety of biological targets such as enzymes (isomerase, oxidoreductase, flavoenzymes), proteins (mitochondrial proteins, microsomal proteins).[3,4,5,6] The benzoquinone core is therefore an interesting scaffold for the design of a library of chemical probes to be used in chemical genetics.

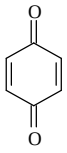

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Figure 1: *Structure of 1,4-benzoquinone **1** scaffold.*

The concepts of chemical genetics will be introduced and background will be provided for the use of benzoquinone as a natural and synthetic product. The goals of this thesis will then be presented. Finally, the results of the synthetic and biological work will be presented and discussed.

1.1 Chemical Genetics

In 2004, the sequencing of the human genome was completed.[7] There were high expectations that this would accelerate our understanding of cellular processes at the molecular level, thereby aiding in the development of novel therapies for disease. Alone though, the genetic information from the genome sequence is not enough to comprehend intricate biological mechanisms: the link between genes and their functions needs to be established. Modern genetic methods have made it possible to rapidly identify genes and their mutant alleles by simple database operations. Gene cloning and knockout techniques allow the overexpression or silencing of proteins in lower organisms such as fruit flies (*Drosophila melanogaster*), zebra fish (*Danio rerio*) and mice (*Mus musculus*) to produce observable phenotypical effects. Complementarily, human models based on cell lines can also be engineered using molecular biological methods. Although developments in genetics have advanced the understanding of biological processes, there are still some limitations: the study of essential genes is prevented because organisms with mutations in such genes are not viable. Furthermore, genetic approaches are not well suited to studying dynamic cellular processes that occur on time scales of minutes or seconds.

An alternative approach to linking genes and proteins to their function and phenotypes is termed chemical genetics, and uses small molecules to perturb protein networks of biological systems.[8] It is a multiple step approach that generally begins with the assembly of a collection of small molecules, followed by screening of the compounds in a developed assay and finally ends with the identification of the modulated target molecule. Like classical genetics, this approach attempts to uncover the specific macromolecules (usual proteins) that act as regulators of cellular processes. Their functions are subsequently defined using protein biochemistry, molecular cell biology and synthetic chemistry.

In analogy to genetics, chemical genetics can be divided into two alternative approaches namely: forward and reverse chemical genetics (*Figure 2*).[9] These approaches will be briefly described in the following sections and will be illustrated with specific examples.

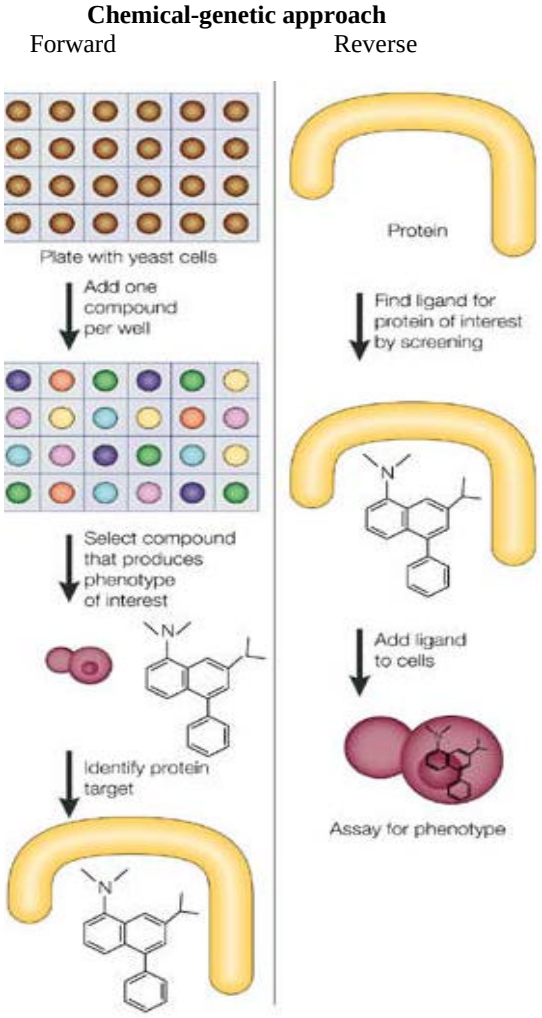

Figure 2: Summary of forward and reverse chemical genetics approach to understanding biological systems.[7]

Forward chemical genetics involves the use of small molecules to screen for a desired phenotypical effect on the biological system under investigation. Once a small molecule has

been identified, its molecular target needs to be determined. This can be achieved by using molecular cell biology and protein biochemistry.

For example, Mayer *et al*. used a combination of two phenotypical assays for screening of a 16,320 member compound library for compounds that affect mitosis.[10] One synthetic small molecule, monastrol (**2**), provoked the reorganization of the mitotic spindle (*Figure 3*).

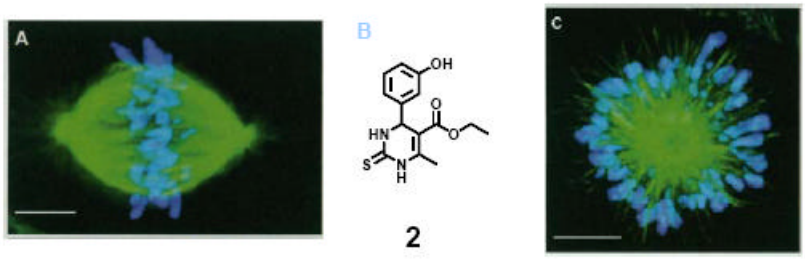

Figure 3: (*A*) cell with normal mitotic bipolar spindle; (*B*) *chemical structure of monastrol* (**2**)*;* (*C*) *reorganization of mitotic spindle visualized by microscopy*.[7]

The phenotype induced by monastrol had been observed before on inhibition of the mitotic kinesin protein Eg5 using anti-Eg5 antibodies.[11,12] The effect of monastrol is reversible: removing the compound by washing allows cells to move out of mitotic arrest and complete mitosis normally. This property was used to study the function of Eg5, which is now an established cancer target.[13]

In reverse chemical genetics, a small molecule is identified against a selected purified protein. This molecule is then used to "knockdown" the protein in question at the cellular and organismal level.

For example this approach was used to screen a library of compounds to find a small molecule that binds and inactivates the protein MEK1, an enzyme whose activity is needed for cell division.[14] A potent and selective MEK inhibitor PD184352 (**3**, *Figure 4*) was identified (50% inhibitory concentration, IC_{50} 17 nM).

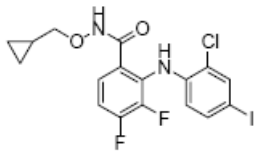

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Figure 4: Chemical Structure of PD184352 (**3**).

In order to define the function of MEK1 in cell cycle progression, cell growth and cell morphology, the effect of PD 184352 was subsequently studied in vivo on mice with colon carcinomas of mouse and human origin. These experiments demonstrated that tumor growth was inhibited by up to 80 % upon treatment with PD184352. The low toxicity, high potency and selectivity made this a promising compound for the treatment of colon cancer.

Particular advantages of chemical genetics over classical genetics are that temporal control is possible as small molecules can be added to the studied systems at any time point during the experiment. The effects are also reversible as the compounds can be removed metabolically or by washing. In contrast to achieve reversibility in a genetic system, conditional alleles need to be introduced and these are normally difficult to generate and control. Another advantage is that small molecules have rapid effects, as they are mostly diffusion limited. They can therefore be used to observe immediate effects. Furthermore, they can be used to study critical genes in developmental stages: whereas a cell knockout may not be viable, it may still be possible to study the effects of a knockdown gene product.

However, the main disadvantage is that chemical genetics cannot be applied always straight forward. Any gene, in principle, can be specifically manipulated by genetics; chemical genetics still needs to find selective small molecules. In forward chemical genetic studies the protein-targets still need to be uncovered, which at present is still a challenge.

The use of small molecules to study biological systems has a long tradition. The more recent systematic use in the context of chemical genetic approach has had a strong impact on fields such as signalling[15,16], cell morphogenesis[17,18], and developmental biology.[19]

1.1.1 Application Areas of Chemical Genetics

The use of small molecules to affect biological phenomena, also known as chemical genetics has made a significant impact in diverse areas of biology:

Developmental biology

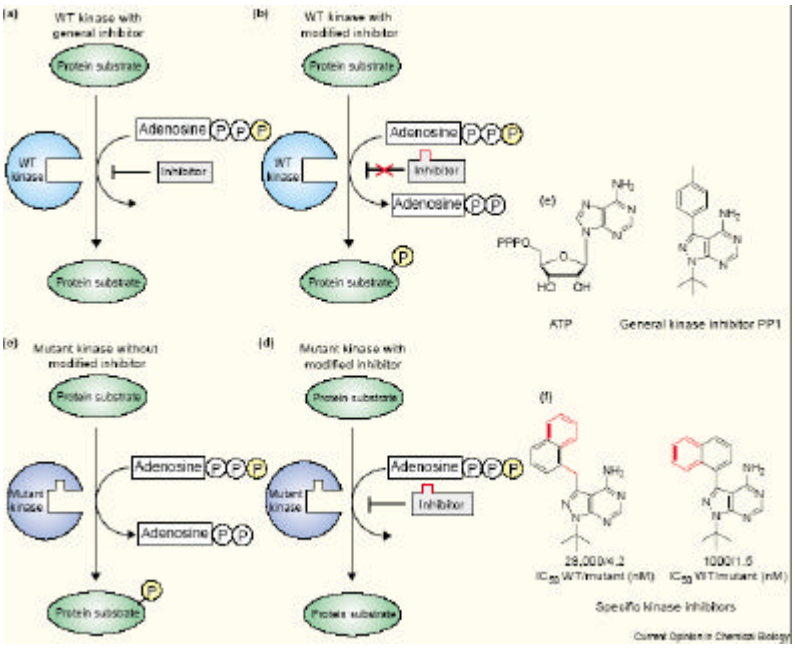
In developmental biology many studies have involved the use of the chemical genetic approach. The advantage of using chemical genetics in this field is that it is possible to control the timing of the addition of compounds. An example of the use of chemical genetics in developmental biology is the control of the sonic hedgehog (Shh) signalling pathway by the alkaloid, cyclopamine. The Shh pathway is implicated in several developmental processes including craniofacial development in embryos, organogenesis in the gastrointestinal endoderm and limb development. Cyclopamine is a naturally occurring compound isolated from *Veratrum californicum*, the ingestion of which by pregnant sheep has severe effects on the developing lamb. Cyclopmanine has been use in the study of Shh signalling underlying organogenesis. Lack of Shh signalling in gastrointestinal endoderm results in the formation of presumptive pancreatic tissue, whereas adjacent tissue expressing Shh gives rise to the stomach and duodenum. Forced expression of Shh pathways was found to block normal pancreatic development. This observation led to the hypothesis that the use of cyclopamine to inhibit Shh synthesis is critical for rendering gastrointestinal endoderm competent for pancreas development.[20]

Signalling

Kinases

Kinases are enzymes with a crucial role in transmitting signals between cells and inside cells. They are involved in a variety of important cellular functions. Kinases work by phospohorylating proteins, which are then activated and able to perform specific functions. They control the activation of proteins that cause disease and therefore are prime targets for drug development. They play a significant role in nearly all-cellular signalling pathways as well as in metabolic disorders, cardiovascular diseases, cancer inflammation, autoimmune disorders and neurological diseases.

This large family of homologues enzymes has been difficult to study by either chemical or genetic techniques. Because the ATP-binding site is highly conserved throughout the kinase family it is difficult to find small molecules of suitable specificity for chemical studies, and knockouts fail to account for "compensation", where other kinases can compensate for the absence of a specific kinase during the development process of the organism. By combining the features of genetics and chemistry, Shokat *et al*. developed a general approach for regulating with small molecules the kinase enzyme activity in yeast.[21,22] Using site-direct mutagenesis, they manipulated one of the kinase genes, removing a hydrophobic residue with glycine or alanine and creating a new pocket in its ATP-binding site. This did not affect the kinase activity (*Scheme 1, c*), but changes the ability of the enzyme to bind different ligands. In this way they found that small molecules that bind in this new site and inhibit the mutant protein do not affect any other kinase protein (*Scheme 1, b).*



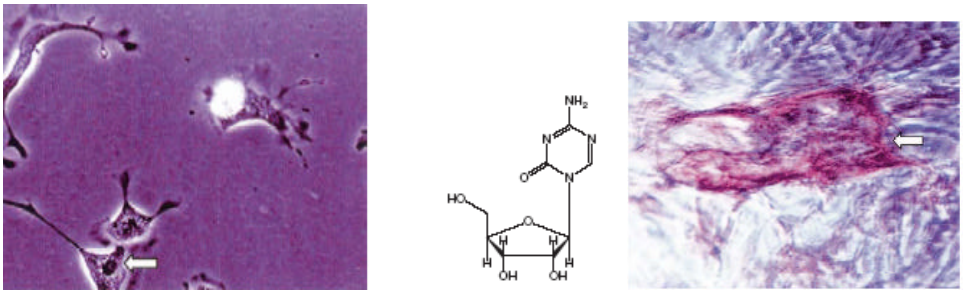
Scheme 1: Kinase-specific inhibition achieved using chemical genetic reported by Wood et al.[13]

a) Wild-type kinases inhibited by a non-specific inhibitor. b) Wild-type kinases are not inhibited by non-specific inhibitor analogs containing a sterically bulky fuctional group. c) Engineered kinases show normal kinase activity. d) Engineered kinases are inhibited by inhibitor analogs with sterically bulky functional group. e) Chemical structure of ATP and the general kinase inhibitor PP1. f) Chemical structure of specific kinase inhibitors structure in red indicates modified group.

Transcription

Chemical genetics has been used in many studies involving the interaction of ligands with nucleic acids. One example is the use of chemical genetics to study the role of DNA methylation during the cell differentiation process. DNA methylation is implicated in epigenetic control of gene expression and is believed to play a significant role in cell lineage commitment during development. The role of DNA methylation during the cell differentiation process has been demonstrated through use of 5-aza-cytidine (*Figure 5b*), a nucleotide analogue that induces DNA hypomethylation. It has been shown that the adult mesenchynal

stem cells isolated from fatty tissue are differentiated into cardiomyocytes after treatment with 5-azaC.[23] The transformed cells exhibited the multinucleated morphology (*Figure 5a*), contracted spontaneously, and express myosin heavy chain, α-actinin (*Figure 5c*), and troponin-I. These results lead to the observation that DNA methylation is involved in cardiomycite cell lineage commitment.



a) b) c)

Figure 5: Treatment of mesenchynal cells with 5-aza-cytidine. a) Cells exhibiting multinucleated morphology (arrow). b) Chemical structure of 5-aza-cytidine. c) Cells expressing a-actinin (arrow).[23]

Antibiotics

The optimism that accompanied the discovery of the first penicillium antibiotics to treat Staphylococcal infections in the 1940s has been tempered by the emergence of bacterial strains that are resistant to antibiotic treatment. Resistence to antibiotics has been monitored to develop rapidly, within one year to one decade of the introduction of the drug to clinical use.[24] Clinically relevant bacteria are not only characterized today by single drug resistance but also by multiple drug resistences, making them a significant public health threats.

Although antibiotics discovery is an example of the succesful mining of natural products for therapeutic use, in general the discovery of novel lead structures is becoming more and more difficult. Many of the antibiotics in current clinical use are second or third generation

modifications of an older scaffold that still bind to the same protein. Therapeutic scaffolds have been developed via two alternative routes: from a natural product or from a synthetic source. Several natural products have evolved to interact with and to be recognized by targets in pathogenic bacteria. They have successfully served as the basis for creating some semi-synthetic variants of antibiotic natural products currently being used clinically. Antibiotics from synthetic sources include the sulfa antibiotics that target folic acid and the fluoroquionlone family compounds (Ciprofloxacin) that block DNA gyrase and topoisomerase IV. The latest structural class of antibiotics approved for use in this context, the oxazolidinones (Linezolid), was introduced 40 years after the discovery of the fluoroquinolones.

Mechanism of action	**Antibiotic family**
Inhibitors of bacterial cell wall biosynthesis	β-lactams (Penicillium, carbapenem cephalosporin; glycopeptides (Vanomicin)
Inhibitors of protein biosynthesis	Polyketides (tetracyclines); aminoglycosides ; oxazolidinone (Linezolid); ketolides (Erythromycin); macrolides; lincosamides
Inhibitors of DNA synthesis	Quinolones (Ciprofloxacin)
Inhibitors of folic acid synthesis	Sulfonamides

Table 1: Major families of antibiotics and their mechanism of action.

Not only does there appear to be an innovation gap in the generation of novel antibiotic scaffolds, but there are currently only four molecular targets for the main classes of clinically-used antibiotics (*Table 1*): bacterial-cell-wall biosynthesis; bacterial protein biosynthesis; DNA replication; and folate coenzyme synthesis. New targets may be less susceptible to existing mechanisms of bacterial resistance due to a novel mechanism of action.

1.1.2 Library Design for Chemical Genetics

To fully exploit the potential of chemical genetics, it is necessary to have a compound library capable of modulating the function of many proteins. Additional properties of the ideal library include cell membrane permeability and synthetic accessibility.

Not only is it currently impossible to generate a library that contains compounds that bind selectively to each protein, but for many proteins small molecular ligands have not yet been identified. The composition of the library is of great importance, as it should increase the probability of finding interesting substances and effects. Fortunately, many natural products have been shown to interact with a wide assortment of protein and other biological targets. For example, natural products have become effective drugs in a large range of therapeutic indications: a few prominent examples are vancomycin, an antibiotic, paclitaxel, a cytostatic drug used to treat cancer, and cyclosporine, an immunosuppressive agent. Natural products can therefore be considered privileged structures for chemical genetics.[25]

Currently, there are multiple approaches to designing such libraries: libraries based on natural products analogues, libraries based on core scaffolds of natural products and finally libraries based on diversity oriented synthesis. It should be mentioned that these categories overlap to some extent, and that most synthesized libararies are designed to lie somewhere in between.

Libraries based on natural products analogues

This approach involves use of efficient and reliable methods and multistep sequences for the total synthesis of natural products with known biological activity and their analogues on solid support and seeks to establish a structure-activity relationship within the compound class. In this case the substances are biased towards specific targets. One example of the application of this strategy was the synthesis of a 22 analogue library of sarcodictyins (*Figure 6*). Screening of this library lead to the discovery of analogues possessing higher antitumor potencies than

those of the parent structure and also established a structure-activity relationship within the sarcodictycin-eleutherobin molecular framework.[26]

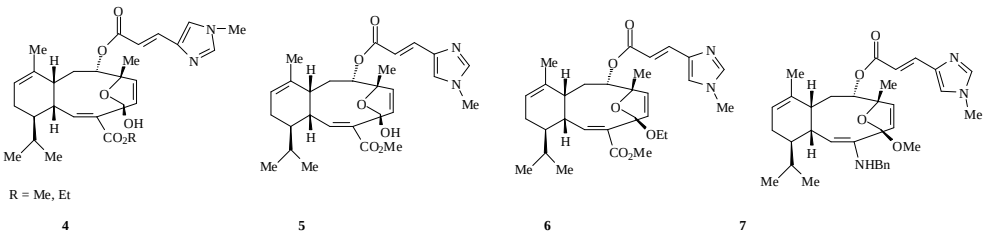
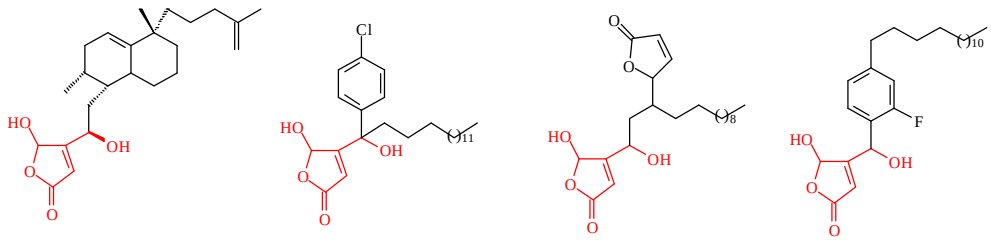


Figure 6: Selected compounds from a sarcodictyin library based the natural product sarcodictyin **(7)**.

Libraries based on core scaffolds of natural products

The second approach is used primarily to exploit the activity of parent natural products. Natural products can be used as biologically validated frameworks upon which to display diverse functionalities. Waldmann and co-workers propose that protein domain cores with similar three dimensional structures can be clustered in so-called protein similarity clusters (PSSC)[27] and that this classification can be used as a guiding principle for the selection of biologically pre-validated starting points for compound library synthesis.

According to this principle, ligands for one of the proteins of the cluster can be used as the starting point to obtain ligands for other members of the cluster. This approach was used to identify inhibitors of the dual-specificity phosphatase Cdc25A **8**, acetyl cholinesterase (AchE, **9**) and 11-β-hydroxysteroid dihydrogenase type 1 and 2 (11-β-HSD-1, **10**, and 11-β-HSD-2, **11**) from a library of butenolides (*Figure 7*).[30]



8 **9** **10** **11**

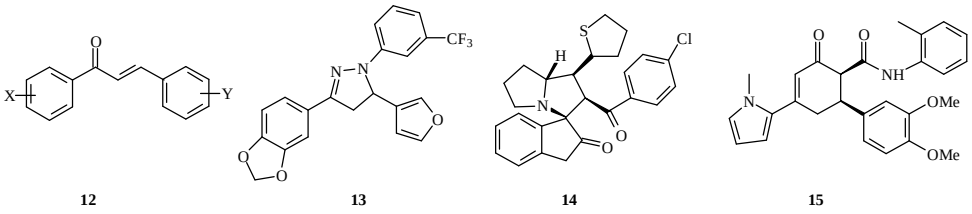
Dysidiolide

Cdc25A inhibitor	AchE	11-β-HSD-1 inhibitor	11-β-HSD-2 inhibitor
IC50 9.4 µM	IC50 1.3 µM	IC50 10µM	IC50 5.3 µM

Figure 7: Selected compounds from a butenolide library based on a core scaffold of the natural product Cdc25A inhibitor Dysidiolide (***8***).

Libraries based on diversity oriented synthesis

This approach seeks to exploit the structural characteristics of natural products in a high-throughput manner: the aims of this strategy are to generate compounds that are easy to access, that have plenty of chiral functional groups and are rich in stereochemical information, and are skeletally diverse.[28] In general, the compounds generated are used as probes for understanding cellular processes. In other words, the substances are not aimed at one particular target. This strategy was applied to generate a chalcone-based library containing 74000 members in nine structural variations (*Figure 8*).[29] Scanning of this library gave hits with a biological activity over a wide range of therapeutically areas, a result that augurs well for discovery screening.



12 **13** **14** **15**

Chalcone structure

Figure 8: Library based on structure of chalcone (**12**).

1.2 Quinones

One class of natural products, the 1,4-benzoquinones has an interesting framework and also biological activity and are therefore good targets for synthesis of a compound collection for the chemical genetics study of their cellular and biological activity. The following sections will describe the biology and chemistry of benzoquinone.

1.2.1 Quinones-Containig natural and synthetic products

The quinone structure (1,4-benzoquinone, *Figure 9*) was recognized as early as the 1930s as a reoccurring structural motif in a variety of synthetic and isolated natural products (*Figure 9*).

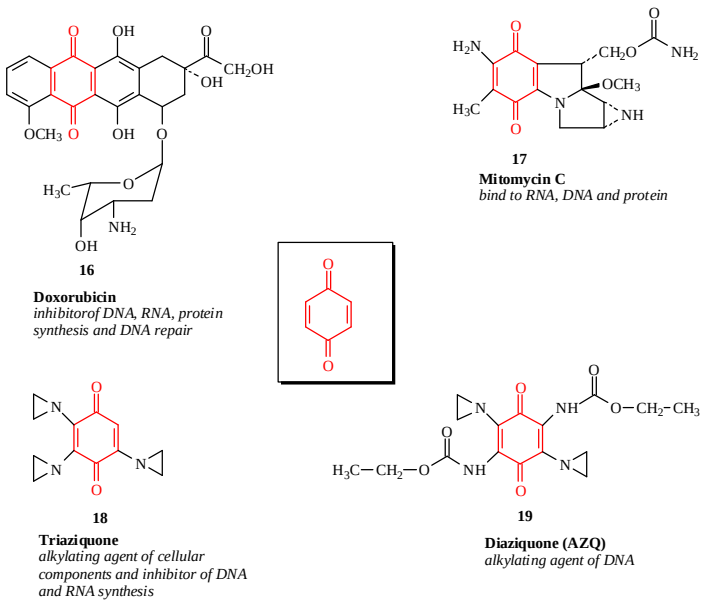


Figure 9: Representative natural and synthetic products containig a quinone motif.

This family of compounds has attracted considerable attention for a number of years due to their broad range of biological activities.[30, 4] A steadily increasing number of quinones are being isolated from natural sources (bacteria, fungi, higher plants, animal kingdom, arthropods and echinoderms) or are synthesized.[3] The biological activity is also remarkably

varied, ranging from antibiotic and anticancer activity, antitumor activity, cytotoxicity as well as playing a role in cellular metabolism.

Doxorubicin (**16**, *Figure 9*) is an antibiotic with antitumor activity from the anthracyclines family that is involved in inhibition of DNA, RNA and protein synthesis as well as blockage of DNA protein-associated strand breaks and inhibition of DNA repair.[31] The target and mechanism of action have been actively studied since 1969[32, 33], and these results have implications for the design of novel antitumor antibiotics.

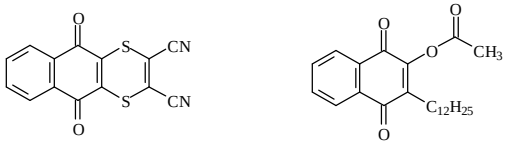
Mitomycin C (**17**, *Figure 9*) isolated from *S.* caespitosis is an antibiotic with high antitumor activity and less toxicity than the other mitomycins from this family, which possessess the ability to bind to DNA, RNA and protein.[34]

Triaziquone (**18**, *Figure 9*) synthesized first in 1958[35] is a benzoquinone-containing alkylating agent with a variety of anti-tumor activities. This agent presumably produces its antitumor effects by alkylation of cellular components[36] as well as by inhibition of DNA and RNA synthesis.[37,38]

Diaziquone (**19**, *Figure 9*) is a cytostatic compound with a significant activity in a wide variety of leukemias, lymphomas and solid tumors.[39,40] Its mechanism of action is not fully understood but appears to be involved in DNA alkylation, crosslinking and strand breaks formation.[41]

Quinones are also found in the agrochemical field as insecticides, fungicides and herbicides. Delan developed at Merck& Co., Inc is a famous fungicide used all over the world today to protect fruits, tree and vegetable against pathogenic fungi.

Acequinocyl from the acaricide family is a regularly used insecticide which acts as a growth inhibitor of many species of agricultural mite at all growth stages as well as an inhibitor of mitocondrial respiration.[42]



20
Delan

21
Acequinocyl

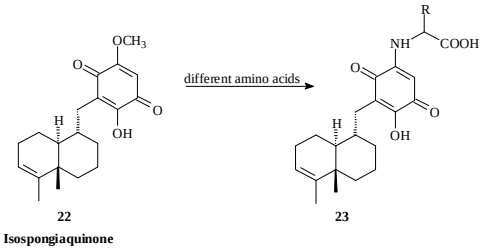
Figure 10: Structure of fungicidal Delan and insecticidal Acequinoscyl.

The interesting biological and structural diversity of this class of substances makes it a particularly interesting template for design of compound libraries in the search for small molecules that affect cellular signalling pathways.

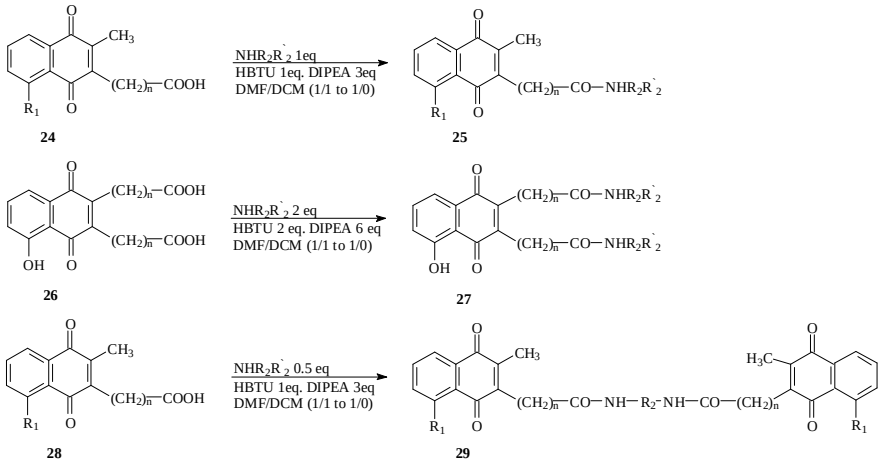
1.2.2 Synthetic routes to a quinone library

Solution-Phase Synthesis of a quinone library

Due the mounting interest in quinones, different libraries were synthesized in solution. Waldmann *et al.*[43] reported synthesis in solution of a nakijiquione library using isospongiaquinone **22** (synthesized previous) as central intermediate for the synthesis of all nakijiquinone analogues. Fifty-six nakijiquinone analogues were synthesized based on the strategy below (*Scheme 2*). Another example is reported by Davioud-Charvet *et al.*[44] They synthesized parallel in solution and on solid phase a library of 1,4-naphthoquinones using different 1,4-naphthoquinone carboxylic acids intermediates **24**, **26**, **28** as building blocks (*Scheme 3*).



Scheme 2: Solution-phase synthesis of nakijiquinone library.



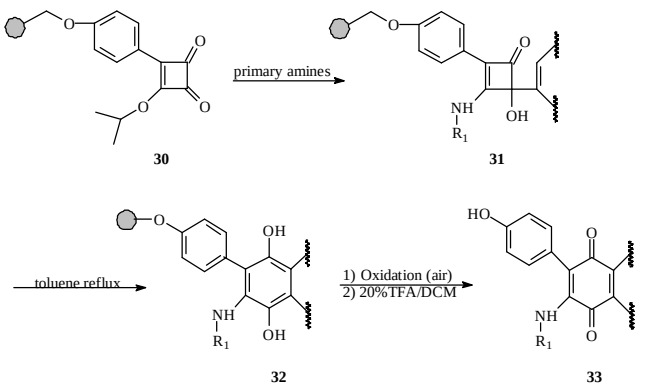
Scheme 3: Solution-phase synthesis of 1,4-naphthoquinones library.

Solid-Phase Synthesis of a quinone library

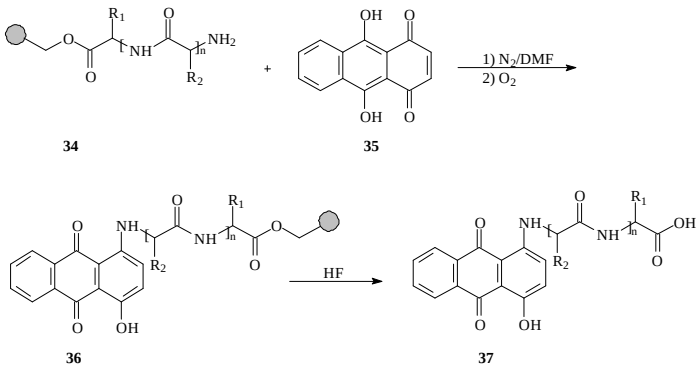
A few methods are reported for solid phase quinone synthesis. These methods are differentiated from one another by the use of different templates and reaction conditions. Armstrong and Tempest[45] have introduced squaric acid as a template for the generation of a quinone library (*Scheme 4*), immobilized on solid phase via a Stille reaction or lithium-halogen-exchange. The resin-bound substrate was used for the synthesis of 1,4-quinone derivatives. In the first step, the isopropoxy-group was displaced with primary amines in a Michael-type reaction. These compounds were then reacted with lithiated aromatic or heteroaromatic compounds or enolethers. After thermolysis and air oxidation in toluene under

reflux, substituted 1,4-benzoquinone derivatives were formed and cleaved from the resin with 20 % TFA/DCM. Unfortunately, the library contains a reduced number of analogues (25) and the reaction yields are low, varying between 0-53 %. Davioud-Chavret *et al.*[44] reported preparation of a library of 1,4-naphthoquinones containing 1360 analogues. In this case the use of 1,4-naphthoquinone carboxylic acid templates provide a library with one variation site.

Finally, Sharma and Gregory[46] reported a 15-member anthraquinone peptide library (*Scheme 5*). The use of Leuco-quinizarin as template directly linked to the amino terminus of the peptide also only affords in this case a library with one variation site.



Scheme 4: Solid-phase synthesis of quinone library using squaric acid template.



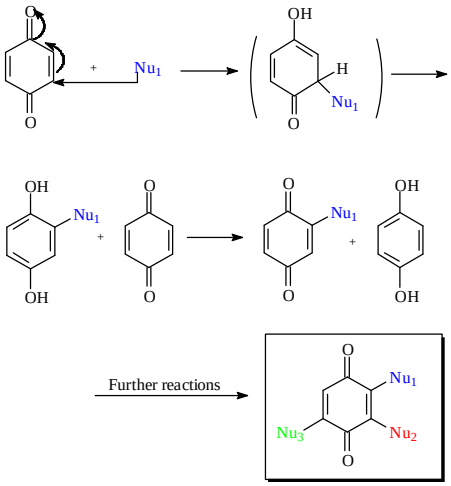
Scheme 5: Solid-phase synthesis of anthraquinone peptides library using Leuco-quinizarin template.

All of the above-mentioned syntheses demonstrate the feasibility of the quinone and quinone precursor as a scaffold for solid phase synthesis.

2 Aim of the Dissertation

The key to the chemical genetics approach is the availability of a highly diverse library with a high percentage of biologically active compounds. The basis and rationale for such a library can be taken from natural products, which are themselves biologically pre-validated structures[47]. An example of such a privileged scaffold is the 1,4-benzoquinone ring system. It is present in numerous natural products that display diverse biological activities[4,37]making it a particularly suitable template for synthesis of a natural-product-based library for chemical genetic studies.

The first aim of this thesis was the development of a rational synthetic route for the synthesis of a *p*-benzoquinone library based on the Michael addition concept. The concept involves regioselective addition of different nucleophiles to the quinone ring with formation of the hydroquinone as intermediate followed in the next step by oxidation to the corresponding quinone. Using the same reaction sequence (addition and oxidation) introduction of different substituents in the modifiable positions of the 1,4-benzoquinone ring is conceivable.



This approach should allow use of a solid support using parallel synthesis methods, simultaneous synthesis of a large number of 1,4-benzoquinone analogues under identical reaction conditions in a systematic manner, so that the products of all possible combinations of a given set of starting materials (building blocks) will be obtained in one reaction sequence.

To demonstrate the feasibility of this strategy and the potential of 1,4-benzoquinone as a scaffold, the synthesis of benzoquinone derivatives should be first undertaken in solution. A successful route for the synthesis of model compounds in solution should open up synthetic pathways for a large number of benzoquinone analogues on solid phase.

In the light of the documented biological activities of benzoquinone natural products, the next objective of this thesis should be the testing of the synthesized benzoquinone library against biological targets.

Although, the combinatorial synthesis can be performed also in solution, for synthesis of the *p*-benzoquinone library, solid phase was chosen for several reasons that include easy parallel

work-up procedures (filtration), accelerated reactions with higher yields by employing excess of reagents and amenability to automation.

3 Results and Discussion

3.1 Synthesis of 2-methoxycarbonyl-1,4-benzoquinone derivatives in solution

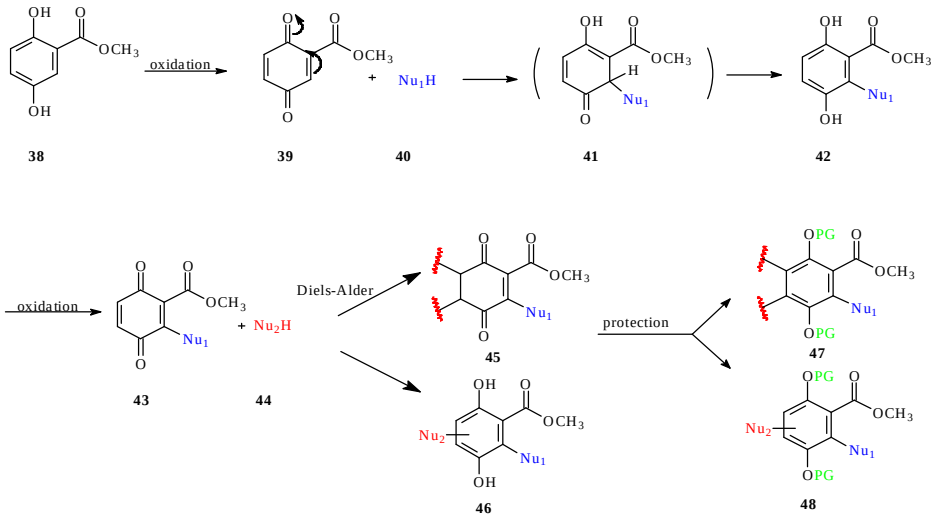
The 2-methoxycarbonyl-1,4-benzoquinone **(39)** was chosen as building block for the synthesis from several reasons. The electron-withdrawing properties of the carbonyl groups play an important role for the reactivity and regioselectivity of the benzoquinone ring and as consequence position 3 and 5 or 6 of the 2-methoxycarbonyl-1,4-benzoquinone **(39)** possess strong electrophilic character and may be selectively attacked by nucleophilic species. [48,49] An additional advantage is that it is easily prepared from commercially available starting materials.

In our studies the 1,4-benzoquinone was also tested as building block for the synthesis. Unfortunately this proved to be incompatible with our concept. First being a symmetrical dienophile offers the possibility of double addition. Second after selective addition of the first nucleophile and oxidation to the corresponding benzoquinone, the scaffold was not suitable for further diversification following the same route. A reasonable explanation could be that after addition of the first nucleophile the quinone ring is electron rich and as result the other position from the quinone ring has low electrophilic character.

Prior to the synthesis on solid phase some model reactions were carried out in solution to check the feasibility of the Michael addition strategy and the potential of 2-methoxycarbonyl-1,4-benzoquinone as scaffold **(39)** as well as different conditions later to be used under solid phase conditions.

3.1.1 General synthetic plan

The synthetic route chosen based on Michael addition approach and provides access to diverse benzoquinone derivatives.



Nu_1= secondary aromatic amines, alcohols, aminoacids.
Nu_2= primary aromatic amines, aromatic thiols, dienes.
PG= acetyl, methyl

Scheme 6: Synthetic strategy towards the synthesis of 2-methoxycarbonyl-1,4-benzoquinone derivatives in solution.

In the first step, selective addition of different nucleophiles **40** in the position-3 of the benzoquinone **39** take place with formation of the monosubstituted hydroquinone **42** as intermediate which is then oxidized to the corresponding benzoquinone **43**. In the following step the resulted monosubstituted 2-methoxycarbonyl-1,4-benzoquinone **(43)** is reacted through an addition reaction with the second nucleophile **44** with the formation of intermediate **45** or disubstituted 2-methoxycarbonyl-1,4-hydroquinone **46** as intermediates.

Finally, protection of the resulted intermediates **45** or **46** gave the desired benzoquinone derivatives **47** or **48**.

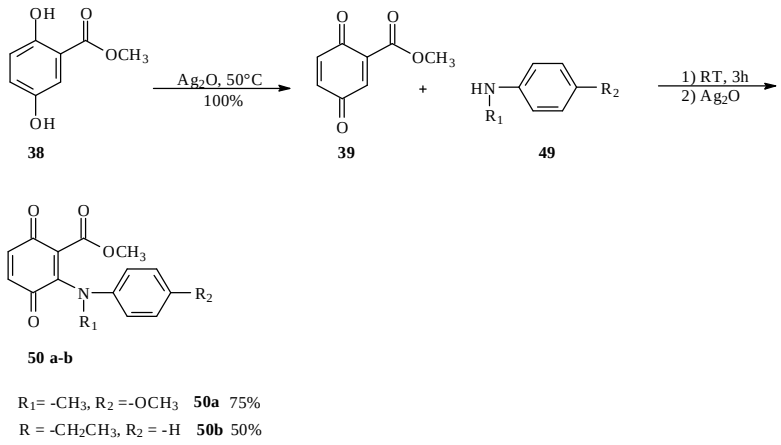
For the synthesis of the diverse 2-methoxycarbonyl-1,4-benzoquinone derivatives, a variety of different nucleophiles were tested as starting materials: aliphatic alcohols, secondary and primary aromatic amines, aromatic thiols, dienes, amino acids and phenols.

3.2 Addition of the first nucleophile to the 2-methoxycarbonyl-1,4-benzoquinone

Selective Michael addition of different nucleophile to the 2-methoxycarbonyl-1,4-benzoquinone ring has been described since long time in the literature.[50,51] For the synthesis of different monosubstituted benzoquinones, nucleophiles with a low nucleophilicity like secondary aromatic amines or alcohols were required. Nucleophiles with a high nucleophilicity like aniline react unselectively.

3.2.1 Addition of secondary aromatic amines to 2-methoxycarbonyl-1,4-benzoquinone

In order to evaluate the possibility to prepare diverse monosubstituted amino benzoquinones by Michael addition different secondary aromatic amines were tested as starting material.



Scheme 7: Synthesis of amino benzoquinones.

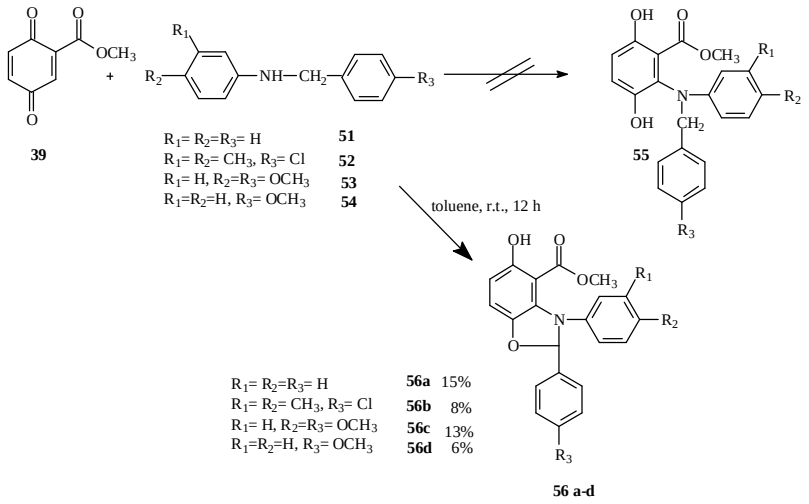
Addition of amine **49** to 2-methoxycarbonyl-1,4-benzoquinone **(39)** is based on the procedure described by P. Müller *et al.* (*Scheme 7*).[51] The reaction involves in the first step oxidation with Ag_2O of 2-methoxycarbonyl-1,4-hydroquinone **(39)** to the corresponding 2-methoxycarbonyl-1,4-benzoquinone **(39)** (90% yield) according to the method described by K. Brunner.[52] 2-methoxycarbonyl-1,4-benzoquinone **(39)** was then reacted with the secondary amine **49** for 3 h at room temperature followed by addition of Ag_2O in order to oxidize the hydroquinone formed in the addition step. Purification by column chromatography gave the desired amino benzoquinone **50 a-b**.

In contrast to the given procedure, the addition was carried out in toluene, the reaction time was 24 h and purification was carried out using toluene/EtOAc as solvent system. The structures were determined by NMR and MS measurements.

Following the same procedure, addition of amines possessing an activated CH_2 group to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** was attempted. Instead of obtaining the corresponding monosubstituted amino benzoquinone, the reaction gave unexpected products and will be discussed in detail in the following chapter.

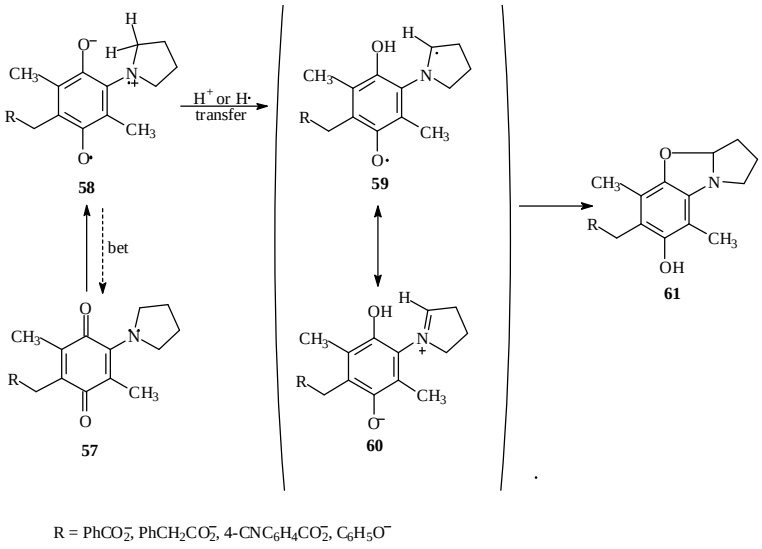
3.2.2 Addition of secondary aromatic amines possessing a CH_2 group

In order to test further the potential of 2-methoxycarbonyl-1,4-benzoquinone **(39)** as scaffold and the utility of the Michael addition strategy, addition of other commercially available secondary aromatic amines was tested (*Scheme 8*).



*Scheme 8: Synthesis of benzoxazolines derivatives **56 a-d** in solution.*

Addition of amines **51**-**54** to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** gave unexpected products: instead of obtaining the expected monosubstituted benzoquinone **55** a novel cyclization was encountered, giving benzoxazolines **56 a-d**, that was then unable to further undergo other transformation. Reactions of this type are known in the literature to take place only under specific reaction conditions. For example, M. G. Steinmetz and Y. Chen reported formation of benzoxazolines from 2-pyrrolidino-1,4-benzoquinone bearing of phenolate leaving groups under photolytic conditions (*Scheme 9*).[53] The mechanism is believed to involve existence of a photoinduced electron-transfer step that would be subject to back-electron transfer (bet) to regenerate the ground state reactant **57**. Electron transfer from pyrrolidino group to the 1,4-benzoquinone moiety in the lowest triplet excited state generate formation of excited state **58** which undergo proton transfer followed by cyclization of intermediate **60** to give benzoxazoline **61**.



Scheme 9: Proposed mechanism for photochemical cyclization of pyrrolidino-substituted 1,4-benzoquinones.[53]

Another example was reported by Eger *et al.* in 2006.[54] They synthesized different dihydrobenzoxazole derivatives (4-amino-emodin derivatives) under strong oxidative conditions (*Figure 11*).

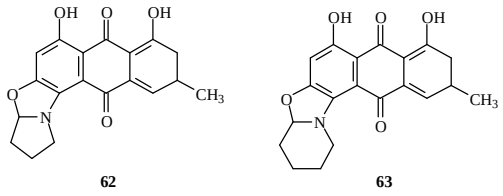
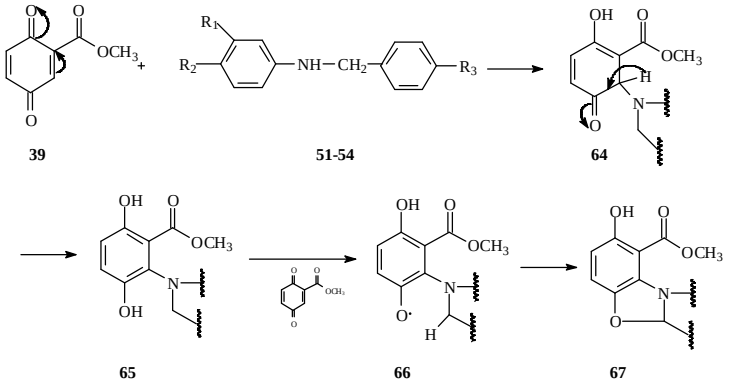


Figure 11: Example of cyclic 4-amino-emodin derivatives.[54]

In the course of this work, the addition of secondary aromatic amines **51**-**54** to 2-methoxycarbonyl-1,4-benzoquinone **(39)** was carried out in toluene, at room temperature and using excess of quinone (2 equiv). The yields vary from 6 to 15 % and are similar to those reported in the literature.[53,54]

A possible mechanism could involve in the first step selective Michael addition of the amine to the quinone ring. In the second step we assume that in this case a second 2-methoxycarbonyl-1,4-benzoquinone **(39)** molecule is used for the oxidation of amine moiety with formation of the corresponding benzooxazole **(67)** as final product through a radical mechanism (*Scheme 10*) . In the literature several examples are known in which oxidation by quinones leads to stable free radicals.[55,56]

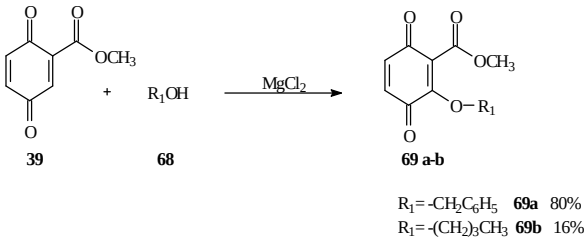


Scheme 10: Proposed mechanism for the benzoxazolines synthesis.

Unfortunately, the addition of this type of amines to the benzoquinone **39** occurs incomplete (unreacted amine could be observed by TLC) and byproducts are formed. Several attempts were done in order to improve the reaction yield. Longer reaction time, addition of different bases like K_2CO_3, DIPEA or the use of unpolar solvent like DCM or EtOAc did not provide better yield.

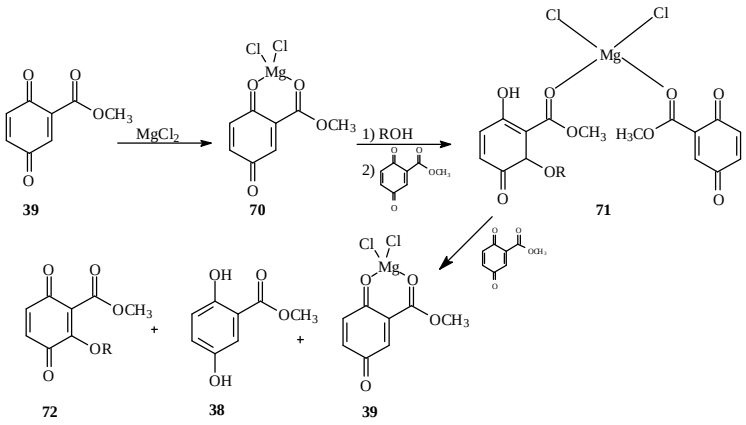
Addition of secondary aliphatic amino acids to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** was first tested on solid phase and will be described in the detail in the Chapter **5.3**.

3.2.3 Addition of aliphatic alcohols to the 2-methoxycarbonyl-1,4-benzoquinone



Scheme 11: Synthesis of 3- alkoxy-1,4-benzoquinones.

Addition of alcohols to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** follows the procedure published by O. E. O Hormi and A. M Moilanen.[57] The reaction involves addition of the alcohol **68** in the presence of $MgCl_2$ as catalyst under N_2 atmosphere to the activated 2-methoxycarbonyl-1,4-benzoquinone **(39)** with formation of alkoxyhydroquinone which is further oxidized by another equivalent of the starting quinone to desired monosubstituted benzoquinone **69 a-b**. The mechanism involves generation of six-membered chelation ring intermediate **70**, followed by subsequent nucleophilic attack of the alcohol to the chelated quinone **70** (*Scheme 12*).



Scheme 12: Proposed mechanism for the addition of the alcohols to 2-methoxycarbonyl-1,4-benzoquinone ***(39)***.

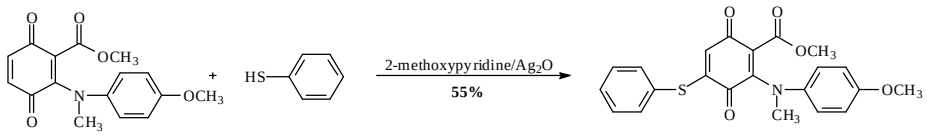
The spectroscopic data (^{1}H NMR, ^{13}C NMR and ESI-MS spectrum) of **69 a-b** are in good agreement with data published by O. E. O Hormi and A. M Moilanen.[57] In the contrast to given procedure, purification was carried out in toluene/EtOAc for compound **69a** and in DCM for compound **69b**.

3.3 Addition of the second nucleophile to the monosubstituited 2-metoxycarbonyl-1,4-bezoquinone

Because the addition of the first nucleophile to the quinone ring causes low electrophilicity of the benzoquinone ring, for the addition of the second nucleophile to the different monosubstituted 2-methoxycarbonyl-1,4-benzoquinones, nucleophiles with a high nucleophilicity and aromatic character are required.

As addition of nucleophiles to the monosubstituted benzoquinones synthesized in the chapter above has until now not been described in the literature, procedures for the addition of other nucleophiles to the 1,4-benzoquinones were used as guidelines.

3.3.1 Addition of thiols to the monosubstituted amino benzoquinones



50a **73** **74**

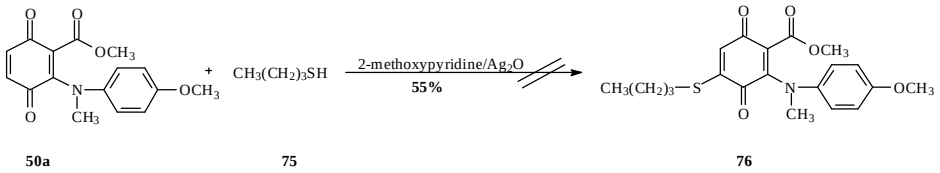
*Scheme 13: Synthesis of 5-Phenylsulfanyl-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(74)**.*

Selective addition of thiophenols to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** has been known for a long time. When the original work of P. Müller *et al.* [51] is considered, thiophenol was reacted with 2-methoxycarbonyl-1,4-benzoquinone **(39)** in presence of 2-

methoxypyridine for 3 h, followed by oxidation with Ag_2O for another 3 h. This procedure leads to the formation of 2-methoxycarbonyl-3-phenylsulfanyl-1,4-benzoquinone.

In the course of this work, the addition of thiophenol **(73)** to 3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(50a)** was carried out using 2-methoxypyridine for deprotonation of thiophenol with concomitant addition of $Ag_2O$ (*Scheme 13*). The reaction leads to the 5-phenylsulfanyl-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(74)** in a 55 % yield.

Following the same reaction procedure, the addition of aliphatic thiols was attempted (*Scheme 14*).

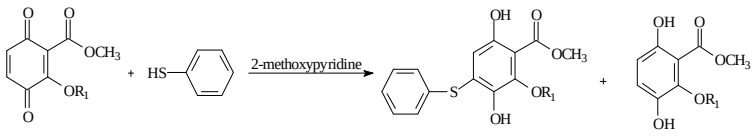


50a **75** **76**

*Scheme 14: Attempt to prepare 5-Butylsulfanyl-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(76)**.*

Unfortunately in this case the desired product **76** was not formed as on NMR spectrum only the starting material could be detected.

3.3.2 Addition of thiols to the monosubstituted alkoxy benzoquinones



69 a-b **73** **77 a-b** **78**

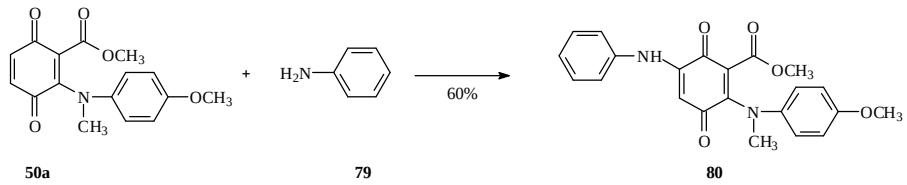
R_1= -$CH_2C_6H_5$ **77a** 16%
R_1= -$(CH_2)_3CH_3$ **77b** 29%

*Scheme 15: Synthesis of 2-alkoxy-3,6-dihydroxy-4-phenyl-sulfanyl-benzoic ester derivatives **(77 a-b)**.*

Addition of thiophenol in the presence of 2-methoxypyridine to the monosubstituted benzoquinone **69 a-b** follows the procedure described by P. Müller *et al*.[51] In contrast to the given procedure, a large excess of thiophenol was used in order to achieve completion of the reaction. However the reaction did not work in the expected yield, a major byproduct isolated was 2-methoxycarbonyl-3-alkoxy-1,4-hydroquinone **(78)** from the monosubstituted benzoquinone **69 a-b**. Attempts were made to optimise the yield. Addition of Ag_2O in order to convert the 2-methoxycarbonyl-3-alkoxy-1,4-hydroquinone **(78)** back to the starting material did not provide a better yield. A longer reaction time was not fruitful either. As usual the structure was assigned by NMR spectroscopy and MS spectrometry.

3.3.3 Addition of amines to the monosubstituted amino benzoquinones

The amines tested were commercially available, and display a range of chemical properties: aromatic and aliphatic.

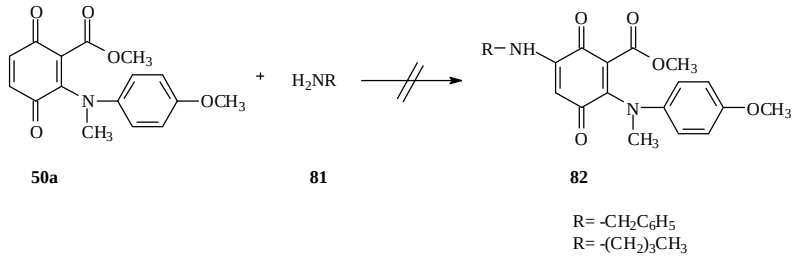


50a **79** **80**

*Scheme 16: Synthesis of 6-phenyl amino-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(80)**.*

Selective addition of aniline to the monosubstituted benzoquinone **50a** has until now not been described in the literature. Synthesis of 6-phenyl-amino-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(80)** involves addition of aniline (excess) to the intermediate **50a** with the formation of 6-phenyl-amino-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-hydroquinone which is unstable at the air and is oxidized to the quinone **80**. A yield of 60 % was obtained after purification. The structure of the product was assigned by NMR spectroscopy and MS spectrometry.

To evaluate the possibility to prepare other disubstituted amino benzoquinones, addition of aliphatic amines depicted below was tested (*Scheme 17*).



50a **81** **82**

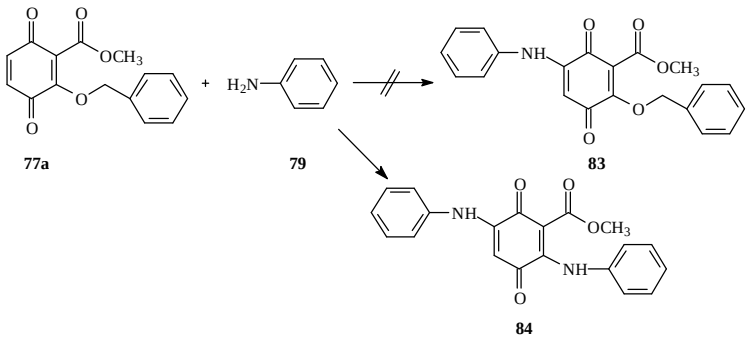
R= -$CH_2C_6H_5$
R= -$(CH_2)_3CH_3$

*Scheme 17: Attempts to prepare 6-amino-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(82)**.*

The reactions were performed as described above using excess of amine in order to achieve completion of the reaction. But none of them were successful. In both cases a complex mixtures of colures products were formed, from which 3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-hydroquinone and unreacted amine was isolated along with some products impossible to be analysed, probably some decomposition products.

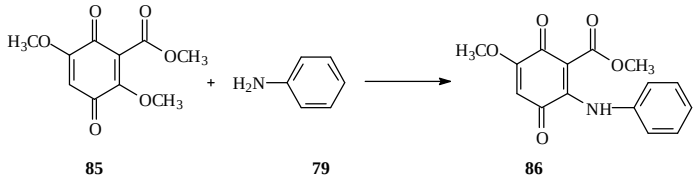
3.3.4 Addition of aromatic amines to the monosubstituted alkoxy benzoquinones

It was described above that nucleophilic addition of aniline to the monosubstituted amino benzoquinones leads to the formation of corresponding diaminobenzoquinones. On that basis addition of aniline **(79)** to the 2-methoxycarbonyl-3-benzyloxy-1,4-benzoquinone **(77a)** was tried. The reaction was carried out in dry toluene and using benzoquinone in excess (*Scheme 18).*



*Scheme 18: Attempt to prepare 6-phenyl amino-3-benzyloxy-2-methoxycarbonyl-1,4-benzoquinone **(84)**.*

However the reaction did not work as expected. After purification by column chromatography using DCM as eluent, two fractions were isolated and analysed by NMR spectroscopy. From these spectra, it was clear that the disubstituted benzoquinone **84** was formed instead of the desired product **83**. Actually, there is in the literature precedence that alkoxy substituent from benzoquinone ring can be substituted by primary amines (*Scheme 19*).[58]

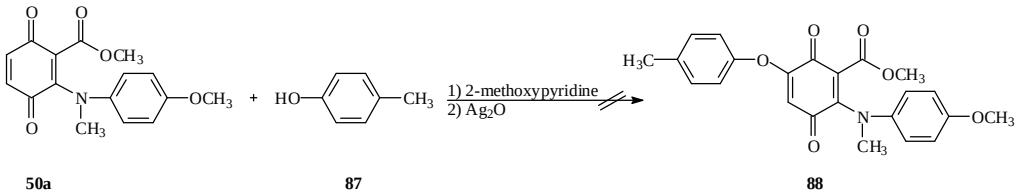


*Scheme 19: Synthesis of 6-methoxy-2-phenylamino-2-methoxycarbonyl-1,4-benzoquinone **(86)**.*

3.3.5 Addition of phenols to the monosubstituted amino benzoquinone

Following a similar procedure as described by *Müller et al.*[51], in which p-cresol was added to the 2-methoxycarbonyl-1,4-benzoquinone, the addition of p-cresol **(87)** to the 3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(50a)** was carried out in dry DCM and in the presence of 2-methoxypyridine as base (*Scheme 20*). After stirring at room temperature for 3 h, Ag_2O was added and the reaction mixture was stirred overnight. From the analysis of the

TLC and NMR spectra, it was clear, that desired product **88** was not formed and only starting material was detected. Somehow the nucleophilic attack did not take place. The problem of this transformation could not be identified. But it might be, that the reactivity of the nucleophile was to low and therefore the addition did not take place.

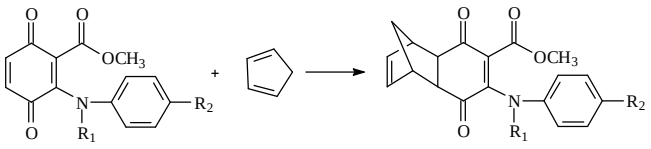


50a **87** **88**

*Scheme 20: Attempt to prepare 6-methylphenoxy-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **(88)**.*

3.3.6 Addition of cyclopentadiene to monosubstituted amino benzoquinones

To further evaluate the possibility to prepare other disubstituted benzoquinone derivatives, cycloaddition to different monosubstituted 1,4-benzoquinones was attempted (*Scheme 21*) using method described by Bäckwall *et al.*[59] as guideline.



50a-b **89** **90 a-b**

R_1= -CH_3, R_2= -OCH_3 **90a** 98%
R_1= -CH_2CH_3, R_2= -H **90b** 98%

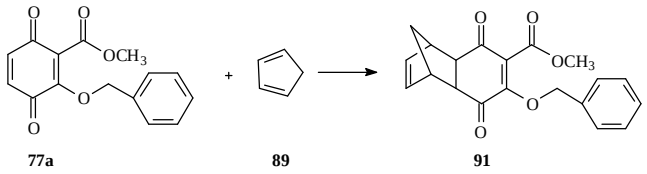
*Scheme 21: Synthesis of 7-(N-methyl-4`-anisidino)-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphthalene-carboxylic-acid methyl ester **(90 a-b)**.*

Compound **90 a-b** was synthesized starting from intermediate **50 a-b**, which was trapped in a Diels-Alder addition with freshly distilled cyclopentadiene **(89)**. Purification by column

chromatography gave the desired product **90a**, respectively **90b** in 98% yield. As usual, the structures were elucidated by NMR spectroscopy and MS spectrometry.

3.3.7 Addition of cyclopentadiene to monosubstituted alkoxy benzoquinone

As in the case described above the main reaction is a Diels-Alder addition between cyclopentadiene and 2-methoxycarbonyl-3-benzyloxy-1,4-benzoquinone **(77a)** (*Scheme 22*). A 95% of desired product **91** was obtained after purification. Structure assignment was provided by NMR spectroscopy as well as ESI-MS spectrometry.



77a **89** **91**

Scheme 22: Synthesis of 7-benzyloxy-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester ***(91)***.

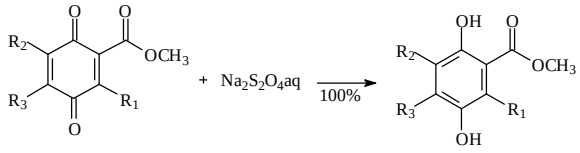
3.4 Protection of the disubstituted benzoquinones derivatives

In order to introduce diversity in the position 2 and 5 of the benzoquinone ring and to prevent later decomposition of the quinone derivatives at the cleavage conditions required by solid phase, protection of the carbonyl groups from the benzoquinone ring was required and will be described in detail in the next Chapters.

3.4.1 Reduction of disubstituted benzoquinone derivatives

The reduction of 1,4-benzoquinones to hydroquinones has long been known. A commonly method uses saturated aqueous solution of $Na_2S_2O_4$ in dichloromethane. This method was used by Osmo E. O. Hormi and Anu M. Moilanen[57] for the reduction of monosubstituted 2-methoxycarbonyl-1,4-benzoquinone **(92)**. Using their method a 100% yield of disubstituted

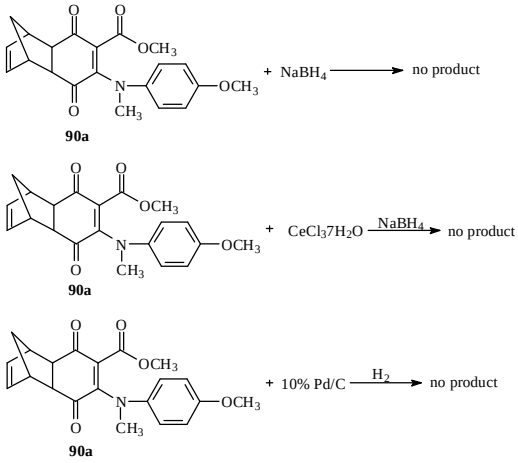
hydroquinone **93** was obtained (*Scheme 23*). The structures were determinate using one- and two-dimensional NMR spectroscopy and by MS spectra.



R_1= -H, R_2= -SC_6H_5, R_3= -$(CH_3)NHC_6H_4OCH_3$ 100% **93a**
R_1= -HNC_6H_5, R_2= -H, R_3= -$(CH_3)NHC_6H_4OCH_3$ 100% **93b**

*Scheme 23: Reduction of disubstituted benzoquinone **92**.*

Reduction of 7-(N-methyl-4`-anisidino)-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphthalene-carboxylic-acid methyl ester **(90a)** proved to be challenging. Reduction to the corresponding aromatizated diols using $NaBH_4$,[60] $CeCl_37H_2O/NaBH_4$[61] or Pd/C[62] was not fruitful due to the instability of the starting material at the reaction conditions (*Scheme 24*).

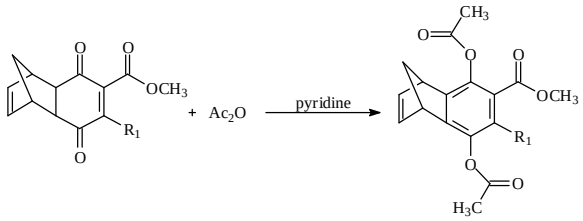


Scheme 24: Attempts to reduce 7-(N-methyl-4`-anisidino)-5,8-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphthalene-carboxylic-acid methyl ester **(90a)**.

Therefore an efficient alternative published by J. Meinwald and G. A Wiley[63] was considered and will be discussed in detail in the next Chapter.

3.4.2 Acetylation of 7-substituted-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphthalene-6-carboxylic acid methyl ester (93)

Following the procedure described by Meinwald and Wiley,[63] intermediate **93** was reacted with Ac_2O in the presence of pyridine as solvent (*Scheme 25*). The reaction proceeds smoothly (7 days). After purification the desired unsaturated diacetate was isolated in a yield varying between 11 and 98%. A plausible explanation for the lower yield in the case of compound **94a** and **94b** could be that the carbonyl groups are sterical hindered by the substituents from the ring. Even, by variation of the reaction conditions (longer reaction time, large excess of Ac_2O) the yield in these cases could not be improved. The structure was assigned using one and two-dimensional NMR spectroscopy as well as MS spectrometry.



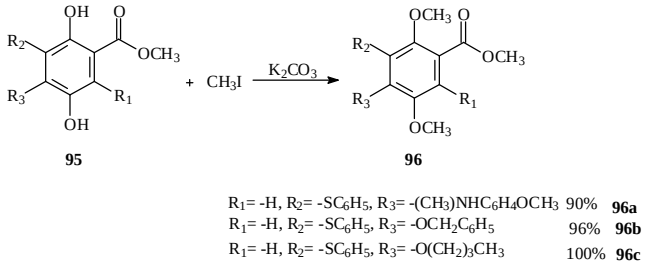
93 **94**

R_1= -$(CH_3)NHC_6H_4OCH_3$	53%	**94a**
R_1= -$(CH_3CH_2)NHC_6H_5$	11%	**94b**
R_1= -$OCH_2C_6H_5$	90%	**94c**

*Scheme 25: Acetylation of 7-substituted-5,8-dioxo-1,4,41,5,8,8a-hexahydro-1,4-methano-naphthalene-6-carboxylic acid methyl ester **(93)**.*

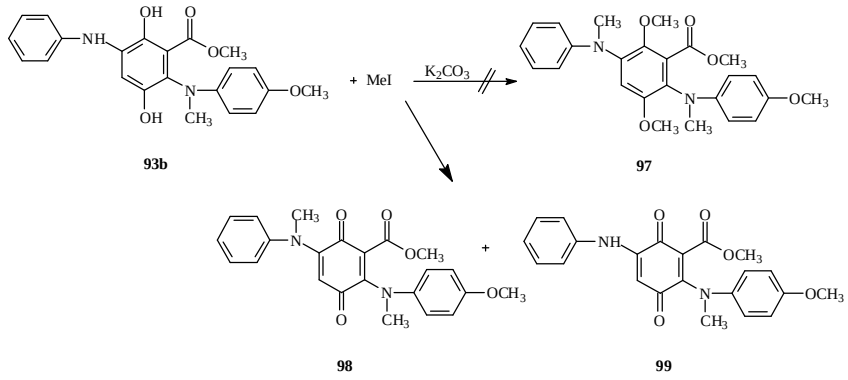
3.4.3 Alkylation of different disubstituted hydroquinones

Similar to the reduction, this is a widely used synthetic step. The method used by T. Tori *et al* [64] was adopted for the protection of hydroquinone **95**. Since phenols are relatively acidic, a weak base (K_2CO_3) is used, suffices to deprotonate them. From a variety of possible solvents, acetone is used as solvent. The alkylation is carried out with MeI under reflux overnight (*Scheme 26*). A yield varying between 90 and 100% of desired product **96 a-c** was obtained.



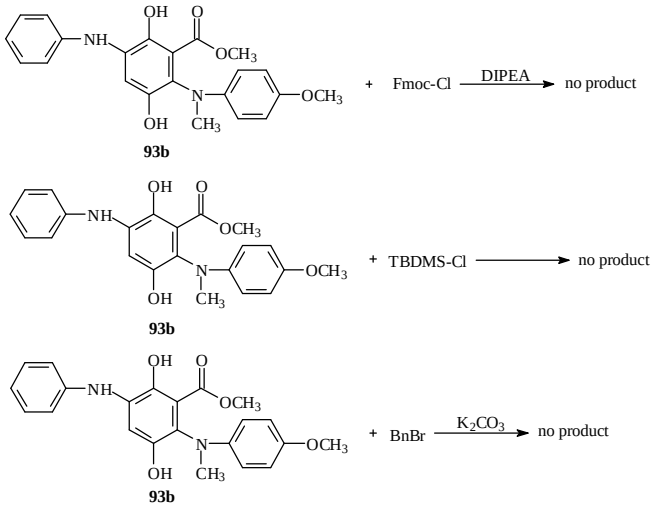
*Scheme 26: Alkylation of disubstituted hydroquinone **95**.*

In the case of 2,5-dihydroxy-6-(N-methyl-4`-anisidino)-3-phenyl amino benzoic acid methyl ester **(93b)** (*Scheme 27*) alkylation did not work as expected, instead of obtaining the desired alkylated product **97**, a mixture of **98** and **99** was obtained.



*Scheme 27: Attempt to protect 2,5-dihydroxy-6-(N-methyl-4`-anisidino)-3-phenyl amino benzoic acid methyl ester **(93b)**.*

A few attempts were to use protecting groups like Fmoc, TBDMS or BnBr, very commonly used for protection of hydroquinones (*Scheme 28*). But none of them were successful. In all cases only starting material was recovered. A possible explanation could be that the hydroxyl groups are sterically hindered by the substituents from the hydroquinone ring and therefore the substitution doesn't take place.



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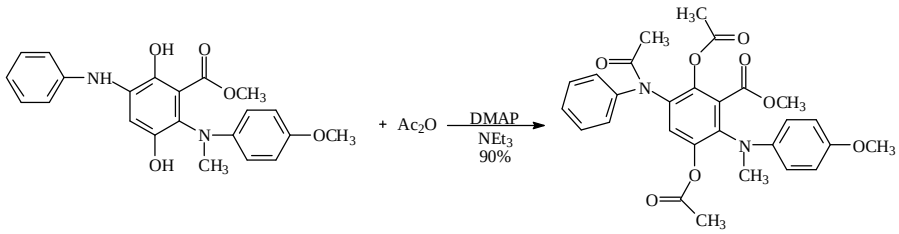
*Scheme 28: Attempts to protect 2,5-dihydroxy-6-(N-methyl-4`-anisidino)-3-phenyl amino benzoic acid methyl ester **(93b)**.*

Therefore the easy and efficient alternative published by G. Höfle *et al.*[65] was chosen as alternative. The acetylation was carried out with Ac_2O, NEt_3 and using DMAP as catalyst *(Scheme 29)*. The reaction leads to the desired product **(100)** in 90% yield.



93b **100**

*Scheme 29: Acetylation of 2,5-dihydroxy-6-(N-methyl-4`-anisidino)-3-phenyl amino benzoic acid methyl ester **(93b)**.*

4 Implementation and Optimisation of the Synthesis on Solid Phase

4.1 Routes for the immobilization of benzoquinone building blocks.

Different synthetic pathways were explored for the immobilization of the benzoquinone to the solid phase. Merrifield resin was chosen as solid support for several reasons. First, it enables the release of the target compound (linked as ether or ester) under mild acidic conditions ($SnCl_4$), which do not affect its stability. Quinone derivatives are known to be sensitive at strong acidic and basic conditions, from this reason the use of other linkers is very limited. Second because the linker must be stable at the reaction conditions required for the synthesis of desired compounds.

Three approaches will be discussed in the following chapters in order to find synthetic routes for the immobilization of benzoquinone scaffold on Merrifield resin.

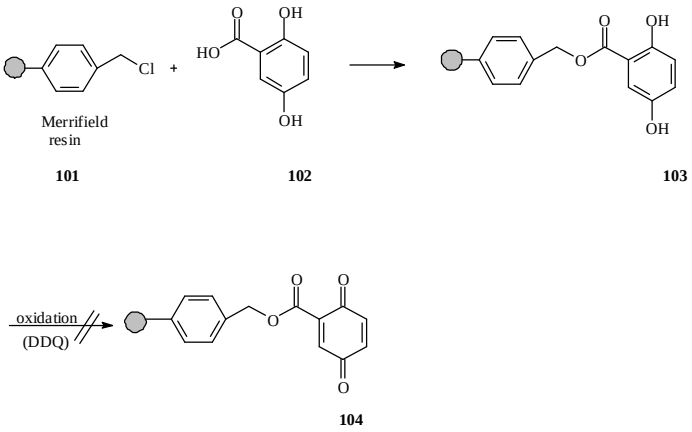
First attempt involves the immobilization of the gentisic acid as ester on the resin followed by oxidation to give desired benzoquinone building block (strategy A).

The second attempt is to immobilize 2,5-dimethoxy-benzoyl chloride as amide on Merrifield followed by oxidation to give the desired benzoquinone building block (strategy B).

The last route describes the immobilization of the 2-methoxycarbonyl-1,4-benzoquinone using nucleophiles as linker to the solid support (startegy C).

4.1.1 Synthesis of benzoquinone building block onto Merrifield resin using 2,5-dihydroxy benzoic intermediate (Strategy A)

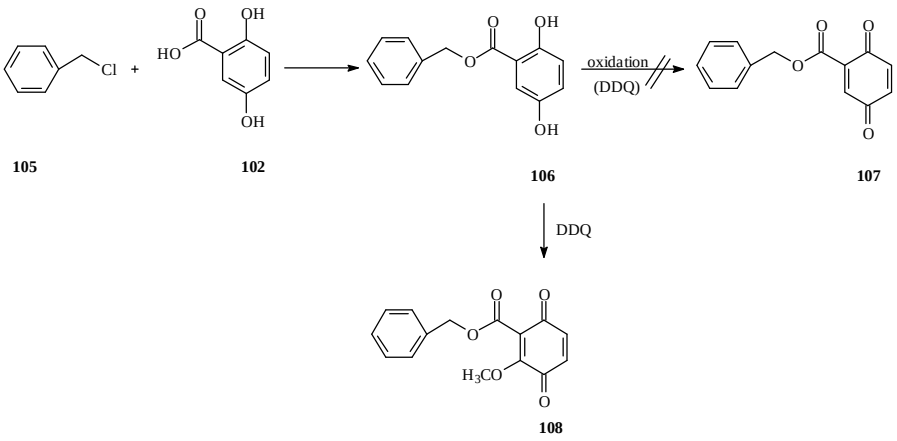
The attempt involves immobilization of the benzoquinone building block as ester to the Merrifield resin and is based on the method described in solution by J. Cavallito and J. S Buck. [66]



101 **102** **103** **104**

Scheme 30: Attempt for synthesis of benzoquinone building block onto Merrifield resin using 2,5-dihydroxy benzoic intermediate ***(102)*****.**

The first step occurs with the loading of 2,5-dihydroxy benzoic acid (gentisic acid) **(102)** onto the Merrifield resin **101** as an ester, followed by the oxidation of the resulted intermediate to the desired building block, benzoquinone **104**. Before performing this approach on solid phase, the synthesis was carried out in solution on a identical model system, 2,5-dihydroxybenzoate (synthesized starting from 2,5-dihydroxybenzoic acid and benzylchloride in the presence of K_2CO_3, 70 °C, 12 h)[68], because the monitoring of the intermediate by cleavage from the solid phase was not possible in this step from instability reasons (*Scheme 31)*.



*Scheme 31: Attempt for oxidation of 2,5-dihydroxybenzoate **(106)** with DDQ.*

Unfortunately several problems prohibit the use of gentisic acid as scaffold for solid phase. First, the oxidation step described in the literature uses an insoluble oxidant (MnO_2), which is not compatible with solid support (just soluble reagents and reactants are compatible with solid phase). Second, replacing of the solid oxidant with a soluble oxidant, DDQ (very commonly used for hydroquinone oxidations)[67], the reaction takes place incomplete and is solvent dependent. In protic polar solvents like MeOH, EtOH, iPrOH the oxidation occurs with the addition of the solvent molecule to the quinone ring and is incomplete (*Figure 12*, ***108***).

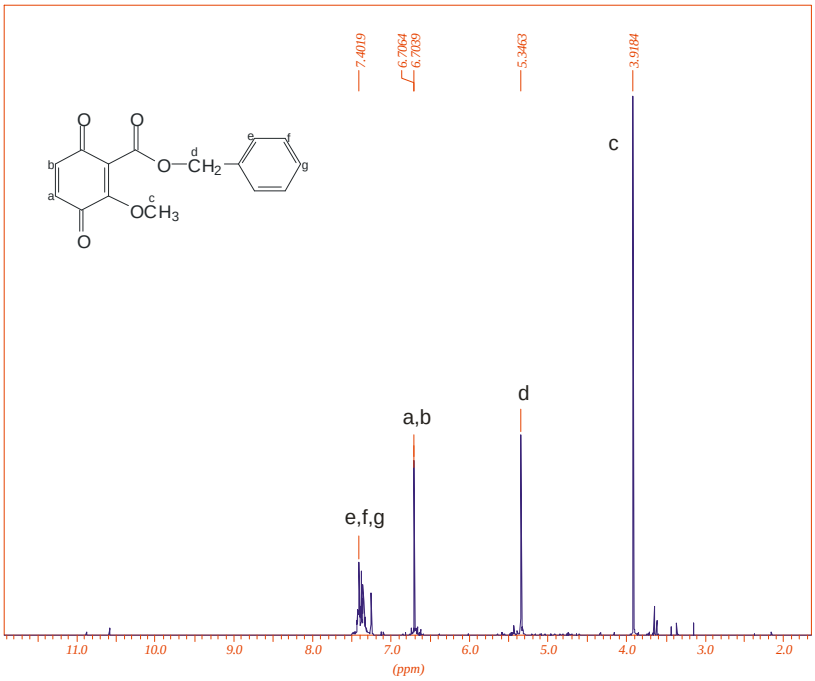
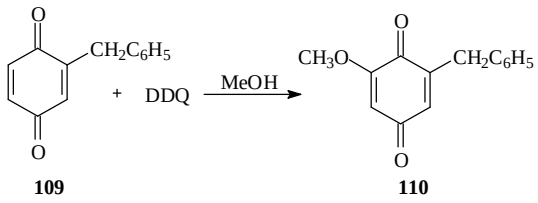


*Figure 12: ^{1}H-NMR of compound **108**.*

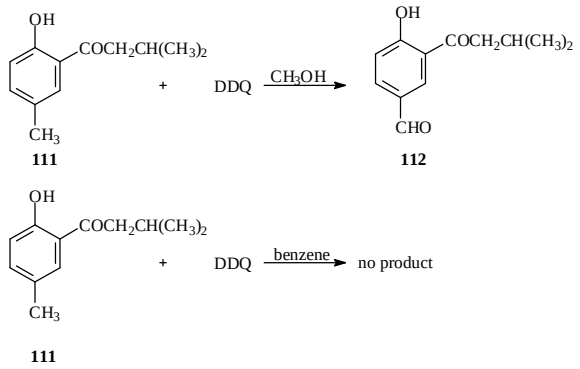
A possible explanation could be that in the first step the oxidation of the hydroquinone **106** to the corresponding benzoquinone take place, followed by a DDQ-catalysed addition of the solvent to the benzoquinone with formation of the methoxy-substituted benzoquinone **107**.

There is in the literature precedence that in some cases oxidation of hydroquinone with DDQ in the presence of MeOH as solvent leads with the formation of methoxy-substituted benzoquinone (*Scheme 32*).[50]



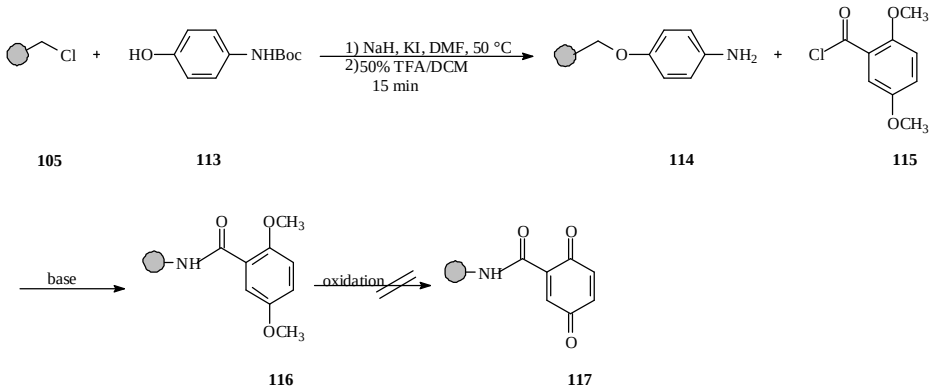
Scheme 32: Oxidation of 2-benzyl-1,4-benzoquinone with DDQ using MeOH as solvent.

In polar solvents like toluene, dioxane, DMF, the dehydrogenation does not take place as only starting material was recovered. There is in the literature precedence that the rate of dehydrogenation is higher in polar solvents than in non-polar solvents, but until now does not appear to be fully understood in which way the course of the dehydrogenation is influenced by the solvent (*Scheme 33*).[50]



Scheme 33: Effect of the solvent on the oxidation with DDQ.

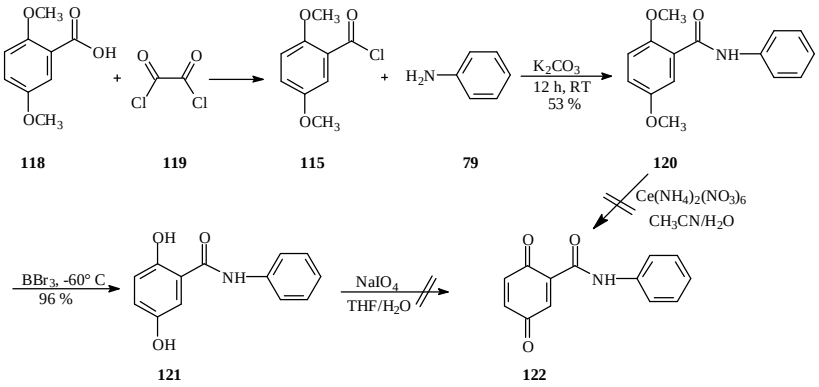
4.1.2 Synthesis of benzoquinone building block onto Merrifield resin using 2,5-dimethoxy benzoyl chloride intermediate (Strategy B)



Scheme 34: Synthesis of benzoquinone building block onto Merrifield resin using 2,5-dimethoxy benzoyl chloride intermediate.

This attempt involves the use of the 2,5-dimethoxy benzoyl chloride **(115)** as starting material for the synthesis of benzoquinone building block. The first step occurs with the coupling of 2,5-dimethoxy-benzoyl chloride (synthesized in solution by chlorination of 2,5-dimethoxy-benzoic acid with oxalyl chloride according to the method describe by Z. Tomaszewski *et al.*[68]) through nucleophilic substitution to the amino functionalised Merrifield resin **105** (synthesized by standard methods, NaH, KI, DMF, 50 °C)[69], followed by subsequent oxidation of the resulted intermediate **116** to the desired building block **117** (*Scheme 34*).

Before performing this approach on solid phase, the synthesis was first tested in solution (from the same reasons presented in the chapter **4.1.1**) using 2,5-dihydroxy-N-phenyl-benzamide **(121)** as model system, which was synthesized in three steps according to the (*Scheme 35*). First step involves synthesis of 2,5-dimethoxy benzoyl chloride **(115)** starting from commercially available 2,5-dimethoxy benzoic acid **(118)** and oxalylchloride **(119)** according to the method described by Tomaszewski *et al.*[68] The resulted intermediate **115** was used immediately in the next step without further purification. In the second step 2,5-dimethoxy benzoyl chloride **(115)** was reacted with aniline in the presence of K_2CO_3 for 12 h at room temperature according to the method described by Bäckwall *et al.*[59] A 53% yield of desired compound **120** was obtained. The last step involves demethylation of **120** in the presence of BBr_3 and -60 °C for 4 h according to the method described by Brimble *et al.*[70] to give the desired **121** in 96 % yield.

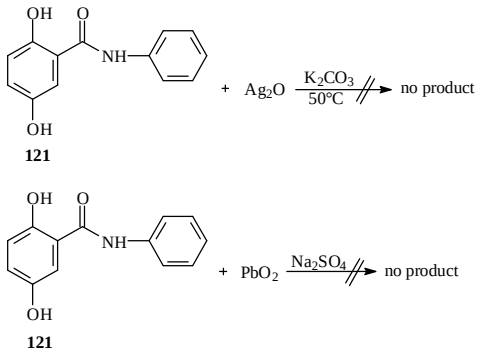


*Scheme 35: Attempts for oxidation of 2,5-dihydroxybenzamide **(122)**.*

Different attempts were done in solution in order to oxidize hydroquinone **121** to the desired benzoquinone **122**. Based on the method described by Parker *et al.*[71] the oxidation of hydroquinone **121** was carried out in THF/H_2O, at room temperature and with 3 equiv. $NaIO_4$. But the desired product **122** could not be isolated. From the analysis of spectra (NMR) only some decomposition products were detected.

The oxidation was also performed starting from the intermediate **120** in the presence of ceric amonium nitrate, in CH_3CN/H_2O solvent at room temperature as described Brimble *et al.*[70] Unfortunately the desired benzoquinone **122** was not formed even after one day. The reaction did not proceed at all and only starting material **120** was recovered.

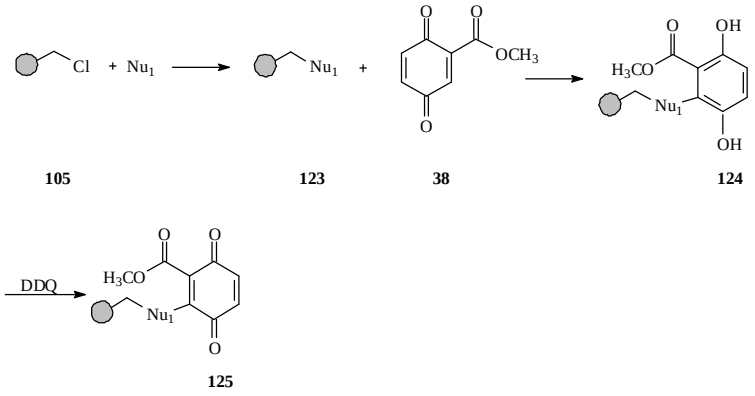
A few attempts were done using solid oxidants as Ag_2O[52] or PbO_2[72] in order to test if the oxidation of the hydroquinone **121** is possible. Unfortunately these were not fruitful either, as only starting material was recovered.



*Scheme 36: Attempts to oxidize 2,5-dihydroxybenzoate **(121)**.*

The problem of this transformation could not been identified. From this reason the strategy was not further pursued and a different alternative was chosen.

4.1.3 Immobilization of 2-methoxycarbonyl-1,4-benzoquinone onto Merrifield resin (Strategy C)



*Scheme 37: Immobilization of 2-methoxycarbonyl-1,4-benzoquinone **(38)** onto Merrifield resin.*

This approach involves in the first step immobilization of different nucleophiles with two functionalities on the solid support. The second step occurs with the immobilization of 2-methoxycarbonyl-1,4-benzoquinone **(38)** through a Michael addition reaction with the

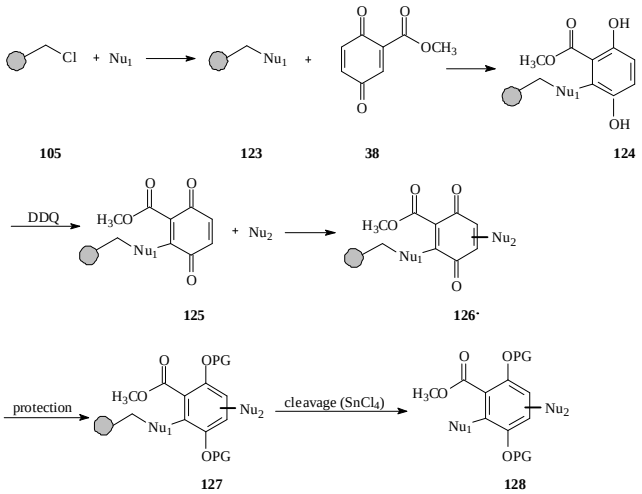
nucleophile linked to the Merrifield resin **123** (as ether or ester) with the formation of hydroquinone **124** as intermediate, which can be easily oxidized with DDQ to the corresponding benzoquinone **125** (*Scheme 37*). In this case the nucleophile Nu_1 serves as linker for the immobilization of quinone scaffold and second as diversity point for library synthesis. As nucleophile Nu_1 are used reactants with a low nucleophilicity because nucleophiles with a high nucleophilicity react unselectively. It must be precise that the intermediate **125** was not cleaved from solid phase in this step due to the instability at the cleavage conditions. Following this approach the immobilization of the quinone scaffold on the solid support was possible and will be discussed in detail in the next chapter.

5 Attempts for the Synthesis of the Benzoquinone Library

To demonstrate the utility of Michael addition method, and that can be implemented in a multistep solid phase synthesis, a challenging multistep synthesis on solid phase was attempted.

5.1 Synthetic plan

The synthetic route chosen is based on the following solid phase approach.

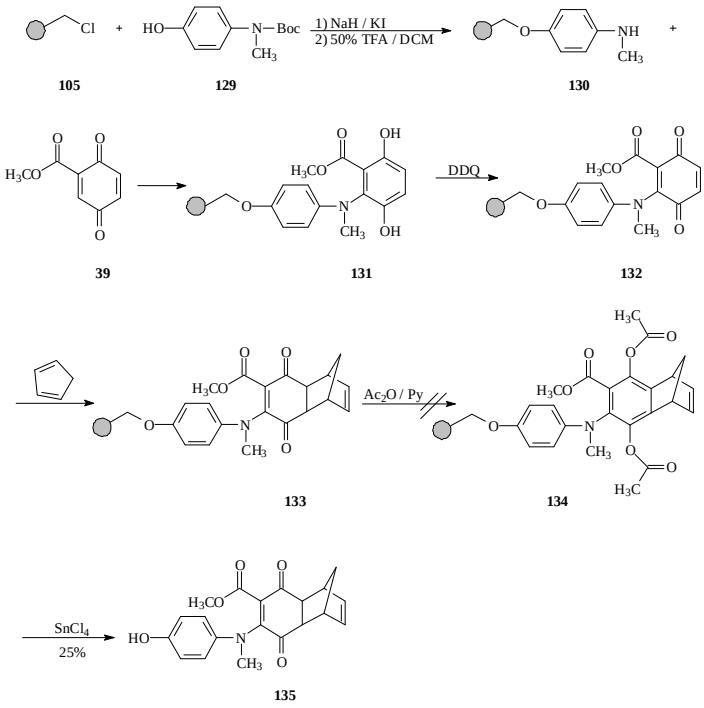


Scheme 38: Synthetic strategy towards the synthesis of benzoquinone derivatives.

The first step occurs with the attachment of Boc protected nucleophiles as ether or ester to the Merrifield resin. Next, 2-methoxycarbonyl-1,4-benzoquinone **(38)** is reacted through an addition reaction with the functionalised resin **123**. Hydroquinone **124** is formed as intermediate, which in the following step is oxidized with DDQ to the corresponding benzoquinone **125**, which is then reacted through another addition reaction with the second nucleophile with formation after the case of the disubstituted intermediate **126**. In the last step intermediate **126** is protected. Finally the cleavage is achieved under acidic conditions ($SnCl_4$) to the desired compound **128**.

By using a solid-phase approach, the time consuming work up steps and the purification of synthetic intermediates are eliminated. This is realized by washing the solid-phase with different solvents; reactions on the solid-phase can be driven to completion by using a large excess of reagents. The cleavage method also ensures the release of the compounds under relatively mild conditions, preventing in this way their decomposition.

5.2 Attempt for solid phase synthesis of 5,8-diacetoxy-7-(N-methyl-4`-anisidino)-1,4-dihydro-1,4-methano-naphthalene-6-carboxylic acid methyl ester (134)



*Scheme 39: Attempts for solid phase synthesis of 5,8-diacetoxy-7-(N-methyl-4`-anisidino)-1,4-dihydro-1,4-methano-naphthalene-6-carboxylic acid methyl ester **(135)**.*

The solid-phase synthesis of 5,8-diacetoxy-7-(N-methyl-4`-anisidino)-1,4-dihydro-1,4-methano-naphthalene-6-carboxylic acid methyl ester **(135)** begins with the loading of Boc-N-methylaminophenol **(129)** (synthesized previous in solution from commercially available N-methyl-aminophenol according to the method described by Fahey *et al.*[73]) on Merrifield resin via a substitution reaction (NaH, KI, over night, 60 °C)[69] providing **130** in a 93 % yield.

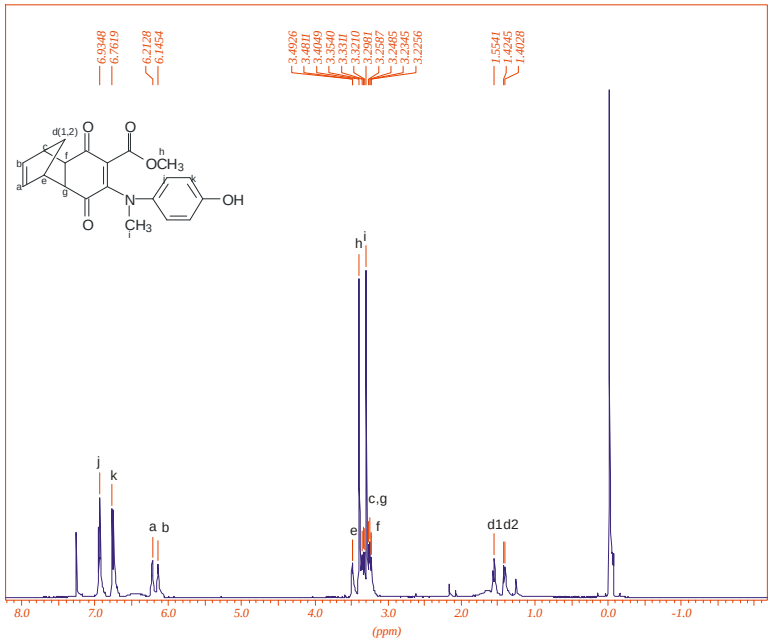
Loading of the resin **130** was calculated by determination of the chlorine left unreacted on the resin using Volhard titration.[74] The method involves addition of an excess of standard solution of silver nitrate to a solution containing the resin. The excess of silver nitrate is then back

triturated with a standard solution of KSCN with ferric iron as indicator in order to calculate the amount of chloride that precipitate with the silver in the first step of reaction.

Following Boc deprotection with 50 % TFA in DCM for 15 minutes, the secondary amine **130** was reacted with 2-methoxycarbonyl-1,4-benzoquinone **(39)** through a Michael addition reaction to give hydroquinone **131** as intermediate which was oxidized in the next step with DDQ to the quinone **132**. Determination of the quinone **132** yield by cleavage was not possible from stability reasons, but the violet colour of the resin indicated that reaction took place. In the next step the second nucleophile, cyclopentadiene was reacted through a cycloaddition reaction with the benzoquinone **132** to afford intermediate **133**, which was in the following step protected with acetic anhydride in pyridine for 7 days.

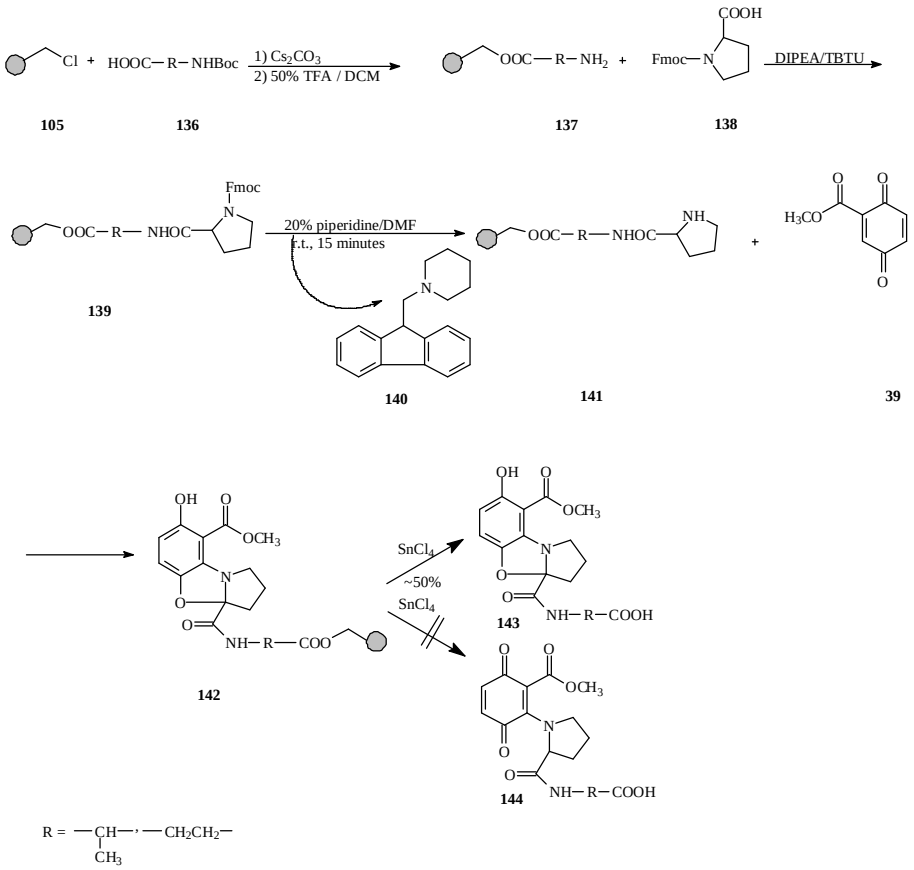
Finally the cleavage from solid-phase was achieved with 10-fold excess of $SnCl_4$ under N_2 atmosphere providing compound **135** in a 25 % yield after purification instead of aromatizated diacetate **134**. Unfortunately, the acetylating step was not successful. This could be due to the solvent, pyridine, which shrinks the resin and diminish, its swelling properties. In order to overcome this problem an attempt was made by using mixture of solvents pyridine/DCM (1:1). Unfortunately in these conditions the reaction did not take place at all even in solution.

A representative ^{1}H NMR spectrum of the cleavage product **135** after purification is shown in *Figure 13:*



To further demonstrate the utility of this strategy and to test the potential of 2-methoxycarbonyl-1,4-benzoquinone, addition of secondary aminoacids was attempted and is presented in detail in the next Chapter.

5.3 Addition of secondary aliphatic amino acids to the 2-methoxycarbonyl-1,4-benzoquinone

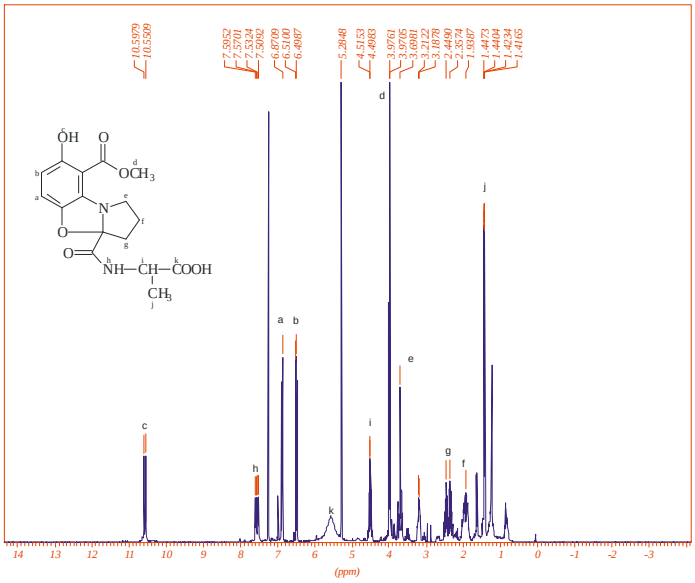


Scheme 40: Addition of proline to 2-methoxycarbonyl-1,4-benzoquinone on solid support.

The first step in the synthetic sequence is the immobilization of N-Boc-amino acid **136** to the chloromethylated resin **105**, which was performed with the appropiate carboxylic acid cesium salt at 50 °C in the presence of KI according to the manufactuers instructions described in Novabiochem catalogue.[69] Then the Boc group was removed by treating the resin with 50 % TFA / DCM (1:1 v / v) for 15 minutes followed by the coupling of the proline **(138)** in the presence of DIPEA and TBTU[75] to provide dipeptide **139**. Analysis of the coupling efficiency

by means of bromphenolblue test[76], which detects the remaining free amino groups, revealed that essentially no underivatized amino groups had remained. In the next step the Fmoc was removed with 20 % piperidine/DMF for 20 minutes to give the desired amino free resin **141**. Loading of the resin was determined by the Fmoc-method[77] with spectrometric monitoring of the absorbance of dibenzylfulvene-piperidine adduct **140**, formed in the cleavage of Fmoc group with 20 % piperidine in DMF for 15 minutes (*Scheme 40*). The resin loadings ranged between 63-92 %.

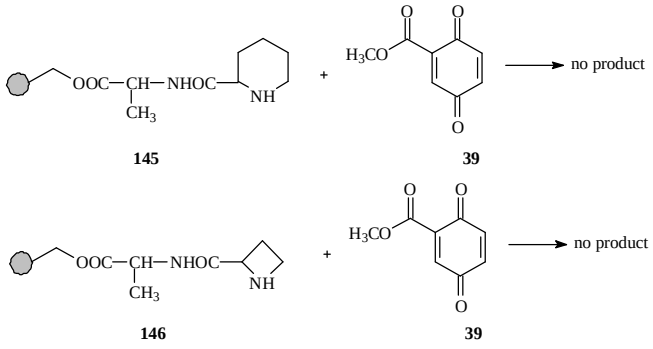
The next step involves addition of 2-methoxycarbonyl-1,4-benzoquinone **(39)** to peptide **141** followed by cleavage with $SnCl_4$.[78] A 50% yield of **143** was obtained instead of the monosubstituted benzoquinone **144**. A representative ^1H NMR-spectrum of the cleavage product **143** directly after workup is shown in *Figure 14.*



In order to compare whether the addition of D- amino acids to the 2-methoxycarbonyl-1,4-benzoquinone gives a better yield compared with the addition of L-amino acids, the same synthesis was employed first using a mixture of D- and L-proline (1:1) and then using D-

proline (*Scheme 40*). In both cases a similar 40-50% yield was obtained. Structure assignment was provided by ^{1}H-NMR spectroscopy as well as by MS spectrometry.

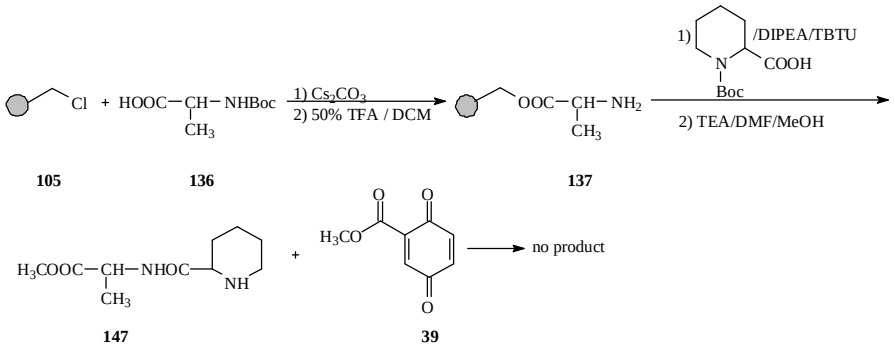
Following the same standard coupling methods (see *Scheme 39*), the addition of the secondary amino acids, N-Boc-2-piperidine carboxylic acid and 1-Boc-L-azetidine to the 2-methoxycarbonyl-1,4-benzoquinone scaffold, was attempted (*Scheme 41*) .



*Scheme 41: Attempts for the addition of **145** and **146** to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** on the solid support.*

However, these attempts proved to be disappointing as only decomposition products were identified by NMR spectroscopy and MS spectrometry. The reason could be that products are unstable at the cleavage conditions.

For that reason, the addition step was carried out in solution and the reaction was monitored by TLC (*Scheme 42*).



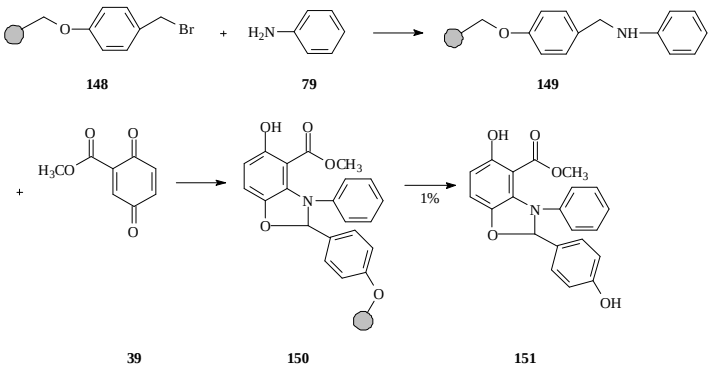
*Scheme 42: Attempt for addition of **147** to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** in solution.*

The reaction involves synthesis of the peptide **147** on solid phase using the standard coupling conditions presented above (See *Scheme 42*), followed by cleavage under basic conditions (TEA/DMF/MeOH, 50°C).[69] The resulted dipeptide **147** was then reacted with the 2-methoxycarbonyl-1,4-benzoquinone **(39)** overnight. Unfortunately the reaction proved to be disappointing as on TLC only decomposition products could be detected.

The problem of these transformations could not be identified, but it might be that the desired product, formed, probably in low yield was decomposed by the air. There are in the literature several examples where benzoxazolines could not be isolated due their instability.[53,54] It was also reported, that is a differentiation of reactivity of five and six membered ring. It was observed, that the formation of the benzoxazoline by photolysis is most efficient with five-membered pyrrolidinoquinone compared with six member ring where just traces of product were detected.[79]

5.4 Synthesis of 5-hydroxy-2-(4-hydroxy-phenyl)-3-phenyl-2,3-dihydro-benzooxazole-4-carboxylic acid methyl ester (151)

In order to further test the feasibility of the strategy presented in the Chapter **5.1**, the synthesis presented in the *Scheme 43* was attempted.



*Scheme 43: Attempt for solid phase synthesis of 5-hydroxy-2-(4-hydroxy-phenyl)-3-phenyl-2,3-dihydro-benzooxazole-4-carboxylic acid methyl ester **(151)**.*

The synthesis of **149** occurs in the first step with the loading of Wang resin **148** by nucleophilic substitution with benzylamine **(79)** according to the method described by M. M Sim and C. W Phon[80] to provide resin **149**, which in the next step was reacted with 2-methoxycarbonyl-1,4-benzoquinone **(39)** to provide the desired resin **150**. Finally the cleavage of final compound from the resin was performed under acidic conditions, with TMSOTf for 3 h according to the method described by H. Yajima *et al.*[81] to afford **151**. However the reaction did not work as expected as only traces of the product (1%) was isolated along with lots of byproducts.

6 Investigation of the Biological Properties of the Benzoquinone Derivatives

The selection of new scaffolds for the generation of chemical libraries is a crucial step taking into account several synthetic requirements, such as compatibility with synthetic technologies and biological relevance. The latter presumes the scaffold to be prone to interaction with biological target-privileged structure. Scaffold taken from natural products are considered to be privileged because of their natural origin. In the next sections the biological properties of the synthesized 2-methoxycarbonyl-1,4-benzoquinone derivatives were investigated using a variety of biological assays as shown in the (*Figure 15*).

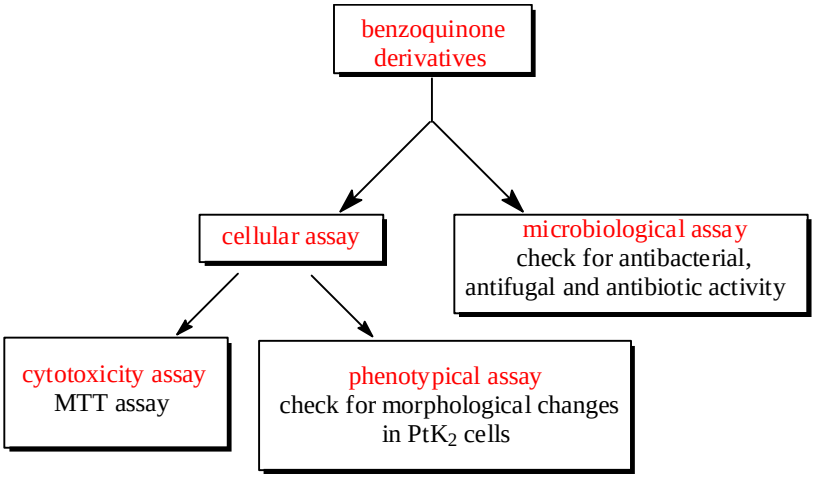


Figure 15: Screening strategy for benzoquinone derivatives.

6.1 Cytotoxicity assay

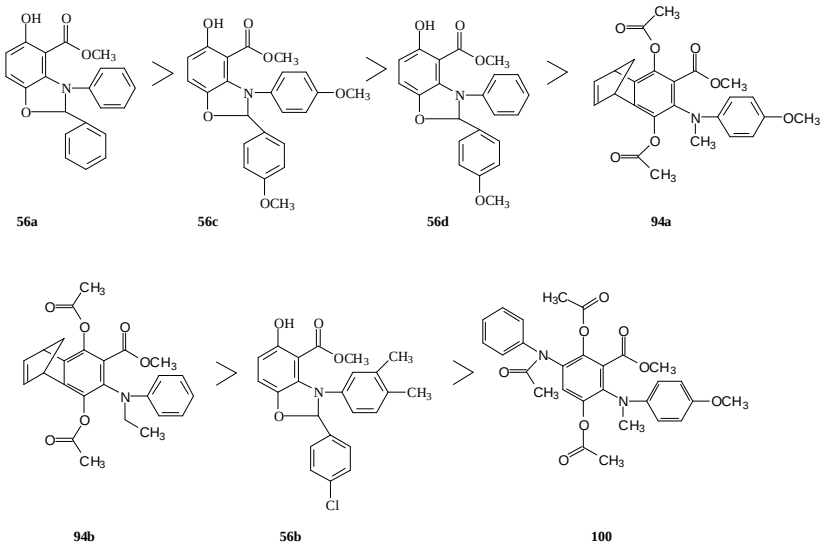


*Figure 16: Structure of cytotoxic compounds **56a**, **56c**, **56d**, **94a**, **94b**, **56b**, **100**.*

When treating cells with compounds, it is necessary to evaluate the effect of the stimuli on the proliferative activity and survival/viability of the cell. These parameters can be effectively monitored by taking advantage of the cell mitochondrion: mitocondrial dehydrogenases reduce tetrazolium compounds inducing colorimetric changes. A commonly used indicator is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).[82] Living cells convert the yellow-colored MTT to a dark blue, water insoluble formazan. For colorimetric determination, the MTT-formazan needs to be dissolved in isopropyl alcohol, which is then measured at 595 nm. The intensity of the signal correlates with the cell count and metabolic activity of the cell.

Figure 17 and 18 show concentration-dependent inhibition curves of the compounds with L-929 mouse fibroblasts. The metabolic activity of the cells grown in microtiterplates was measured by the MTT assay after an incubation period of 5 days. The metabolic activity in

each well is dependent on the number of cells that were grown during the 5 days, but also on their vitality. The absorbance values measured with compound concentrations between 0.01 and 400 µg/ml were related to control wells whose activities were set to 100 %.

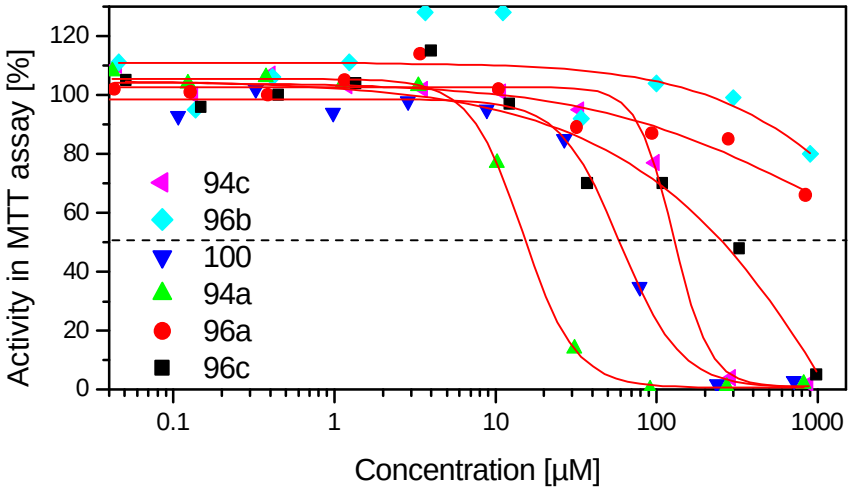


Figure 17: Proliferation of L-929 cells in presence of different concentrations of compounds ***94c, 96b, 100, 94a, 96a, 96c****. MTT reduction activity was taken as growth parameter.*

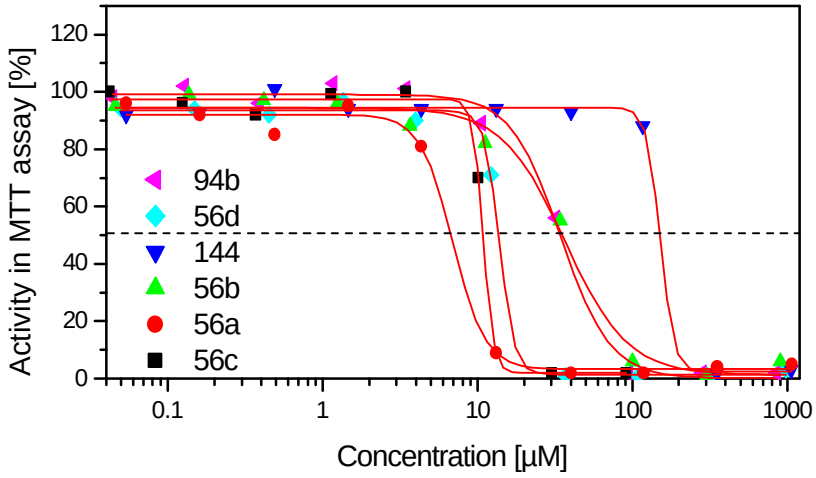


Figure 18:. Proliferation of L-929 cells in presence of different concentrations of compounds ***94b, 56d, 144, 56b, 56a, 56c****. MTT reduction activity was taken as growth parameter.*

From the sigmoid inhibition curves IC_{50} values down to 7 µmol/L **(56a)** were obtained and listed in Table 2.

Table 2. Growth inhibitory activity of compounds **96a, 100, 94a, 94b, 96b, 96c, 94c, 56a, 56b, 56c, 56d, 144** with cultured L-929 mouse fibroblasts.

Compound	IC50	
	[µg/ml]	[µmol/L]
56a	2.3	7
56c	4.5	11
56d	5.1	14
94a	7	16
94b	15	34
56b	15	36
100	31	60
96a	>400	no inhibition
96b	>400	no inhibition
96c	95	231
94c	54	128
144	53	151

Seven compounds (**56a**, **56c**, **56d**, **94a**, **94b**, **56b**, **100**) were identified to have an effect on the cell viability of L-929 mouse fibroblasts cells (*see Table 2*).

6.2 Phenotypic assays

The probability of identifying a selective interaction between a compound and a biological target increases with the number of compound-target pairs assayed. This is the basic concept of empirical high throughput screening (HTS). In the case of a single compound, the number of biological targets available for screening determines the success. A cell is a complex

biological system containing a huge number of target molecules that are all functionally connected: a high content bioassay system. The cell reacts to the action of the compound by developing a distinct phenotype. Thus, cell-based phenotypic screening is particularly effective in the search for bioactive compounds, although the target is not immediately apparent and needs to be identified subsequently. Often, the resulting phenotype is already known and can suggest potential target molecules. Therefore, novel compounds should be investigated for their phenotypic effects on a series of selected cell types.

In order to get more information about the mode of action of benzoquinones immunofluorescence studies with PtK_2 kangaroo rat cells were started. These epithelial-like growing cells are very flat and therefore more suitable for conventional microscopic observations than the L929 mouse fibroblasts because of fewer problems with depth of focus. Derivatives of benzoquinones that show a clear growth inhibition with L929 cells (**94a**, **100**, **94c**, **56a**, **56b**, **56c**) were incubated with the PtK_2 cells first over-night. As we observed that many compounds acted rather rapidly and induced a cell detachment we reduced the incubation time to 4 hours. After fixation the cells were stained for ER and nuclei. **94a**, **94c**, **56a**, and **56b** caused severe changes of the inner structure of the cells. The cells showed large vacuoles (Figure 19B, D), deformations of the nuclei (Figure 19C, D), and vesicles in the ER (Figure 19C, D; Figure 20A,B). In some cases the ER was almost completely dissolved. Especially with **56a** (Figure 20A), but also with **56b** (Figure 20B), the entire ER network was broken down to vesicles only after 4 hours. **100** (Figure 20C) and **56c** (Figure 20D) did not induce such severe damage of the ER system, only wider meshes in the ER network could be observed. The induced changes of the inner membrane structure differ in strength and in detail, but taken together it is difficult to decide whether the phenotypes are due to different mode of actions. The different phenomena seem to overlap.

After 4 hours of incubation with **94c**, single spots of dense ER were observed aside the nucleus, while the rest of the ER network seemed to be unaltered (Figure 21).

Immunofluorescence staining with antibodies against gamma-tubulin, a marker protein of centrosomes, showed that these spots were not connected to the organelles. Their location was independent from these microtubule-organizing centres (Figure 21).

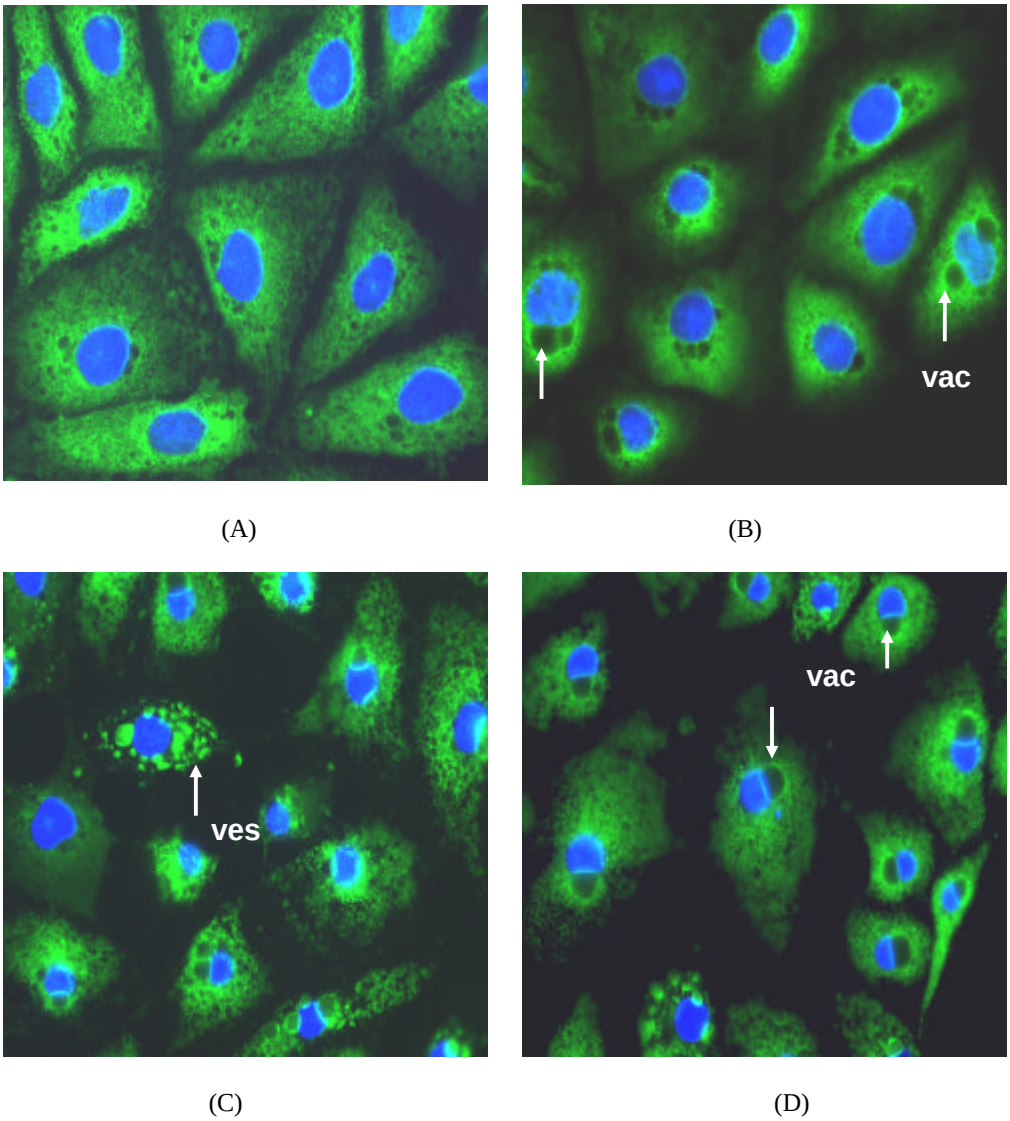


Figure 19: Influence of benzoquinones on cultured kangaroo rat cells (PtK_2). Cells were incubated with methanol (A), ***94a****; 50 µg/ml (B), and* ***94c****; 100 µg/ml (C, D) overnight, fixed and stained for nuclei (blue) and ER (green). Treated cells show deformed nuclei, large vacuoles (vac), and ER vesicles (ves).*

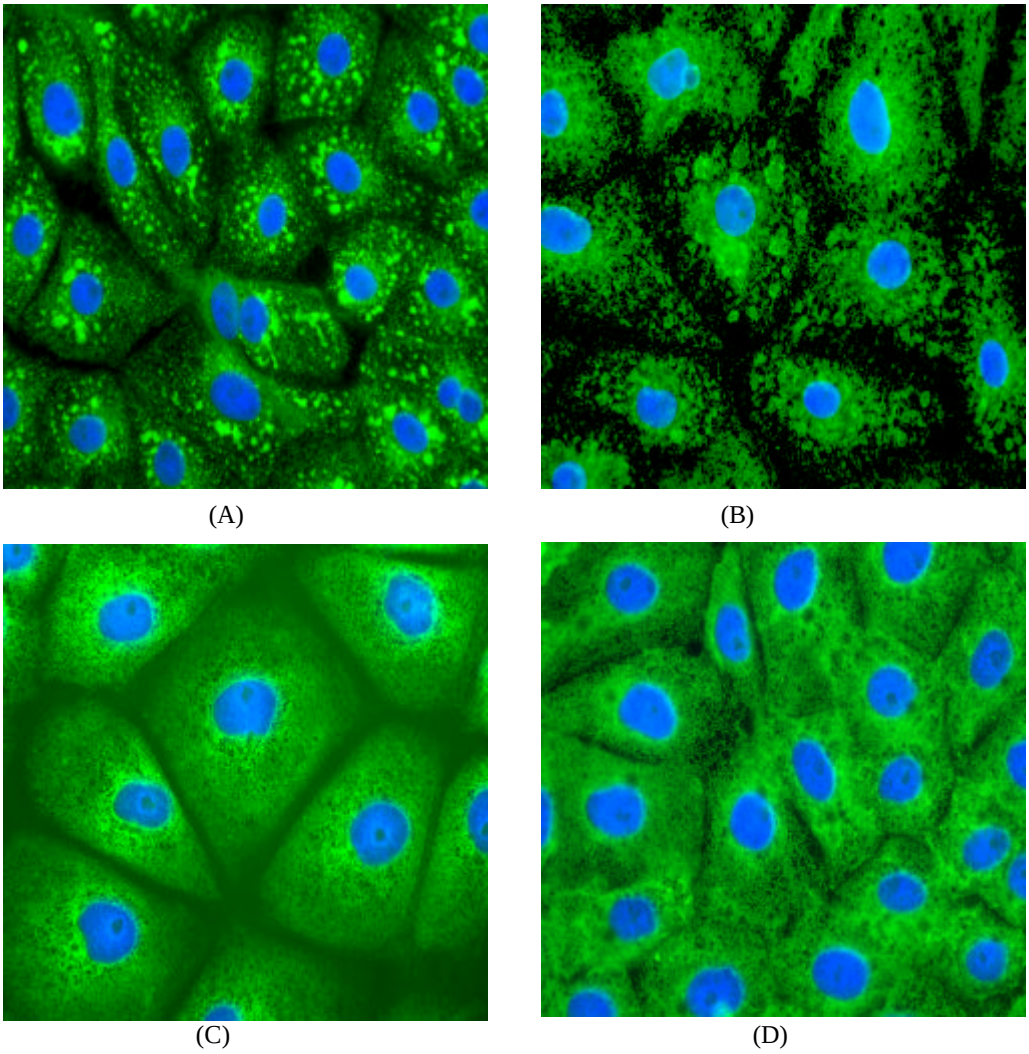
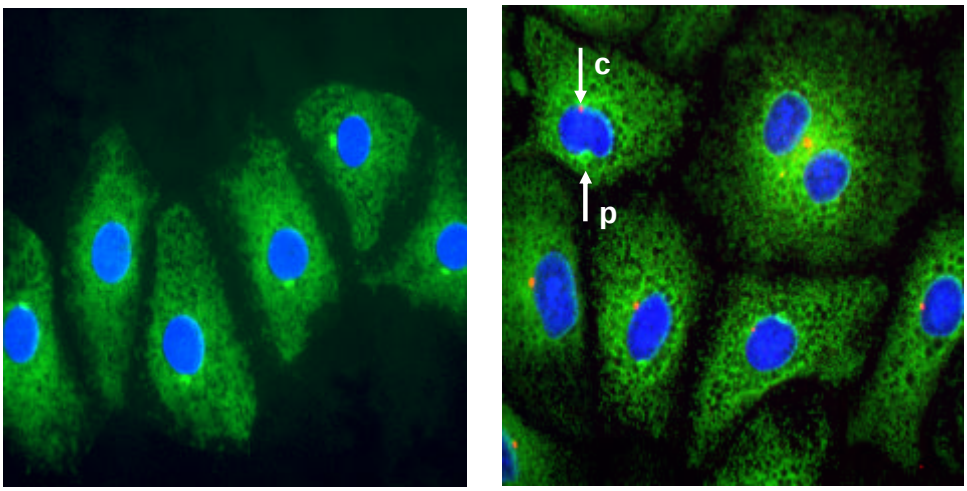


*Figure 20: Influence of benzoquinones on cultured kangaroo rat cells (PtK_2). Cells were incubated with **56a** (A), **56b** (B), **100** (C), and **56c** (D) at a concentration of 100 µg/ml for 4 hours (exception **100** over-night), fixed, and stained for nuclei (blue) and ER (green). The ER structure of cells treated with **56a** and **56b** is totally dissolved to vesicles **100** and **56c** show fewer effects on the ER..*



(A) (B)

Figure 21: Influence of benzoquinones on cultured kangaroo rat cells (PtK_2). Cells were incubated with ***94c*** *(A; 100 µg/ml, B; 66 µg/ml) for 4 hours fixed, and stained for nuclei (blue), ER (green), and centrosomes (red; only in B). The photos show single spots of concentrated ER marker protein (p) near the nucleus. These are not connected to the centrosomes (c)*

Effect on ER structure resembles the action of corallidictyals and siphonodictyols which also contain a benzoquinone moiety (Grube, A., Assmann, M., Lichte, E., Sasse, F., Pawlik, J.R. M. Köck, New bioactive metabolites from Caribbean sponge *Aka coralliphagum*, J. Nat. Prod., in press). These benzoquinones also induce a vacuolisation of the ER. The vacuoles here seem to have a different appearance: they fill the cytoplasm like cushions. A similar effect is also observed with flavonols, *e.g.*, myricetin that induces a vesicle formation in the ER system (Sasse, personal communication).

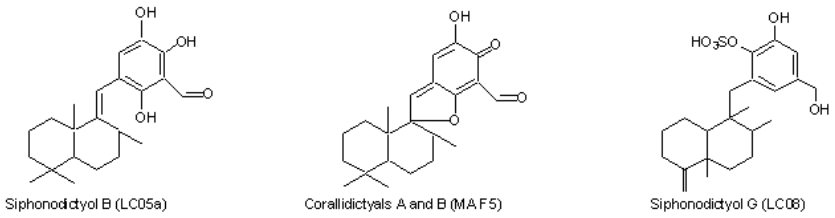


Figure 22: Natural product from Aka coralliphagum

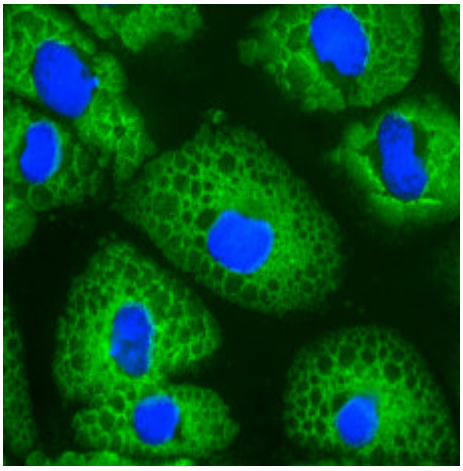


Figure 23: Induction of vacuoles within the ER of PtK_2 cells by corallidictyal.

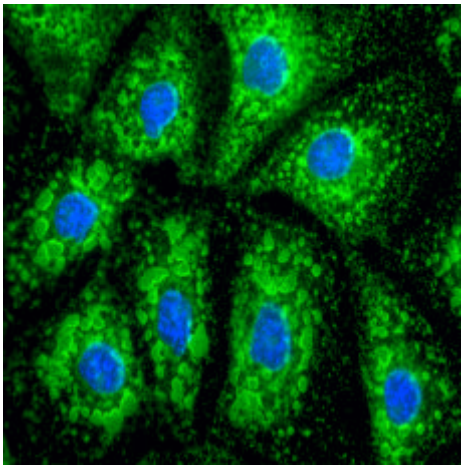


Figure 24: Induction of vesicles in the ER system of PtK_2 cells by myricetin.

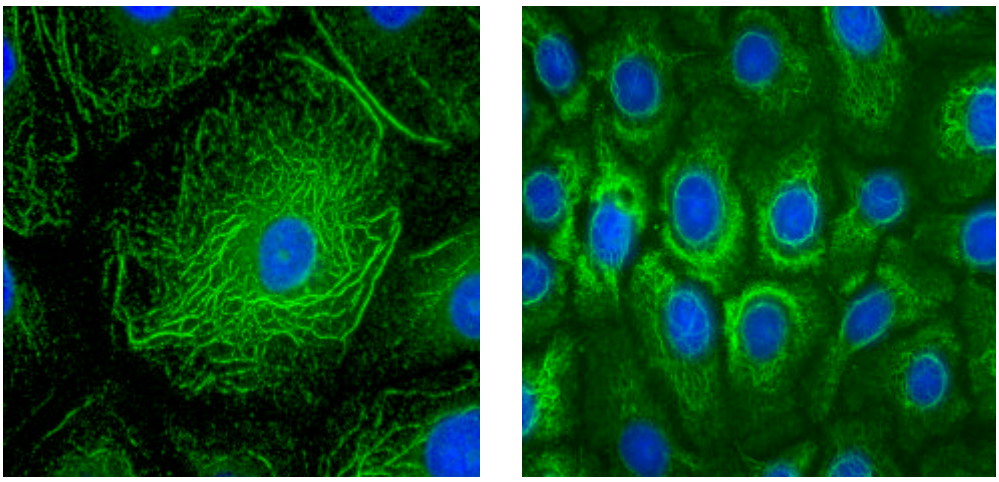


Figure 25: PtK2 cells stained for Golgi zone (green) and nuclei (blue). (A) Control cells; (B) Cells that were treated with ***94c*** *(100 µg/ml) for 4 hours.*

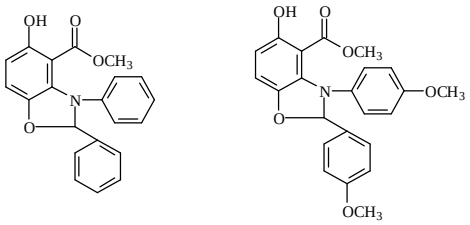
Another hypothesis was that the single spots of dense ER induced by **94c** are due to changes in the Golgi apparatus of the cells. Therefore the Golgi network of treated and untreated PtK_2 cells was labelled by a Golgi zone antibody (*Figure 25*). The control cells show a Golgi network that is concentrated round the nucleus but stretches throughout the cytoplasm. In cells treated with **94c** the Golgi network has a dramatically altered appearance. There are ring like Golgi structures that tightly enfold the nuclei and disordered Golgi structures in the cytoplasm. The network seems to be severely damaged, but there are no structures that could be connected to the observed ER spots.

6.3 Targeting bacteria and fungi

The antibacterial, antifugal and antibiotic activity of the twelve benzoquinone derivatives was determined for *Staphylococcus aureus*, *Mycobacterium phlei*, *Micrococcus luteus*, *E. coli* tolC, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, *Candida albicans*, *Hasenula anomala*, *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Botrytis cinerea* and *Pythium*

debaryanum in agar diffusion assays. The MIC values (in the case of *Saccharomyces cerevisiae*) were used as parameter for antibacterial activity and diameter of inhibitons zones (in the case of agar diffusion assay) were used as a parameter for the antibiotic, antifugal and antibacterial activity.

Only two of the compounds (**56a, 56c)** showed antibiotic activities against *Staphylococcus aureus*, *Mycobacterium phlei* and *Pseudomonas aeruginosa.*



56a **56c**

*Figure 26: Structure of active compounds **56a** and **56c**.*

7 Conclusion

As many benzoquinone-containing natural products have been shown to display diverse and interesting biological activities,[4,37] the 1,4-benzoquinone moiety was chosen as structural scaffold for the design of a natural-product like chemical library for a chemical genetics approach.

With the goal to generate a library of p-benzoquinone derivatives on solid phase via a sequential Michael addition scheme, first a synthetic route was established in solution in order to evaluate the feasibility of this approach and the biological potential of p-benzoquinone.

A set of 11 benzoquinone derivatives was synthesized in solution using 2-methoxycarbonyl-1,4-benzoquinone as building block (*Figure 27*). Diversity was introduced in the addition steps and in the protection step. The synthesis was effected via Michael addition beginning with regioselective addition of the various nucleophiles (Nu_1) to the 2-methoxycarbonyl-1,4-benzoquinone **(39)** with formation of monosubstituted hydroquinone **(42)** which was then oxidized to the corresponding benzoquinone **(43)**. Using the same reaction sequence, addition of the second nucleophile (Nu_2) to the monosubstituted 2-methoxycarbonyl-1,4-benzoquinone **(43)** was achieved with the formation of disubstituted compounds **(45)** or **(46)**. These were then protected by acetylation or methylation to provide the desired quinone derivatives **(47)** or **(48)**.

An exception from the general reaction path was observed in the case of secondary aromatic amines possessing a CH_2 or CH activated group (see *Chapter 3.3.2*), where after addition step, instead of obtaining the corresponding monosubstituted benzoquinone, a novel cyclization was encountered, giving benzoxazolines type **(56a)**, **(56d)** as final product (*see Figure 27*).

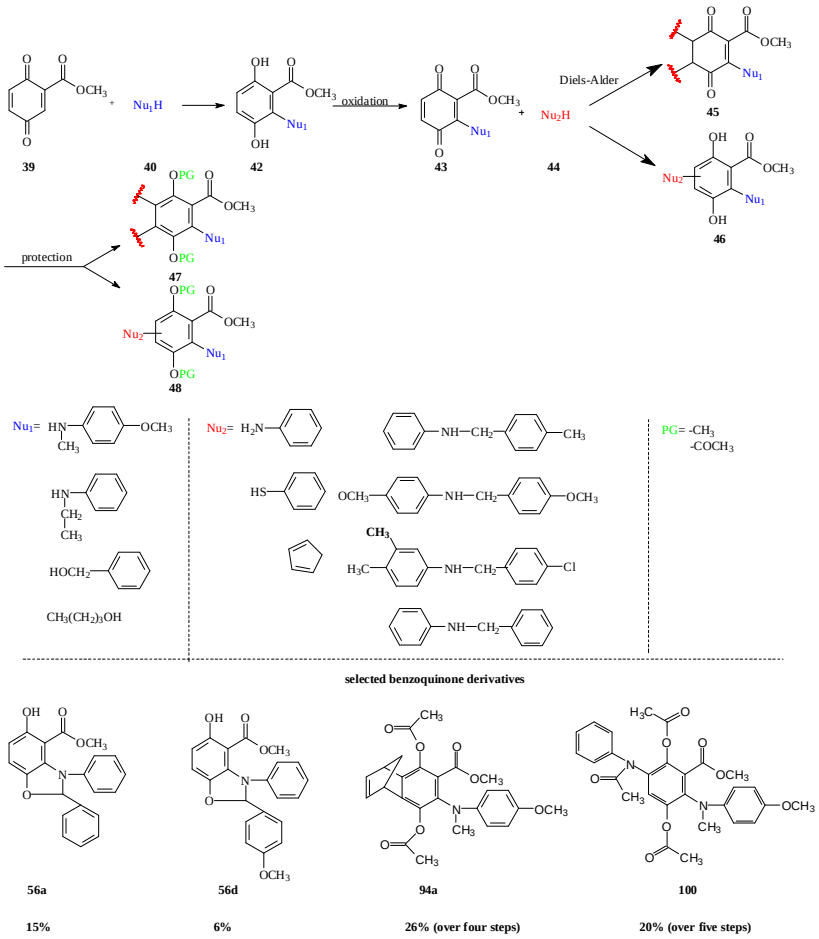


Figure 27: Summary of the synthesis of 2-methoxycarbonyl-1,4-benzoquinone derivatives in solution.

In order to test diversification possibility of the 2-methoxycarbonyl-1,4-benzoquinone scaffold, different classes of nucleophile were tested. Generally, nucleophiles with low nucleophilicity and aromatic character were required for monoaddition, where nucleophiles with high nucleophilicity yielded the diaddition (see *Figure 27*).

In both cases addition of aliphatic nucleophiles proved to be disappointing ending with the mixtures impossible to be analysed. In the last step protection was achieved by acetylation or methylation. Overall, the yields varied from 6 % to 65 %.

The collection of model benzoquinone derivatives synthesized in solution was then used to test their biological activity. In the first study, the benzoquinone derivatives were tested for cytotoxicity. Seven compounds were cytotoxic beginning at a concentration of 60 μM (*Figure 28*).

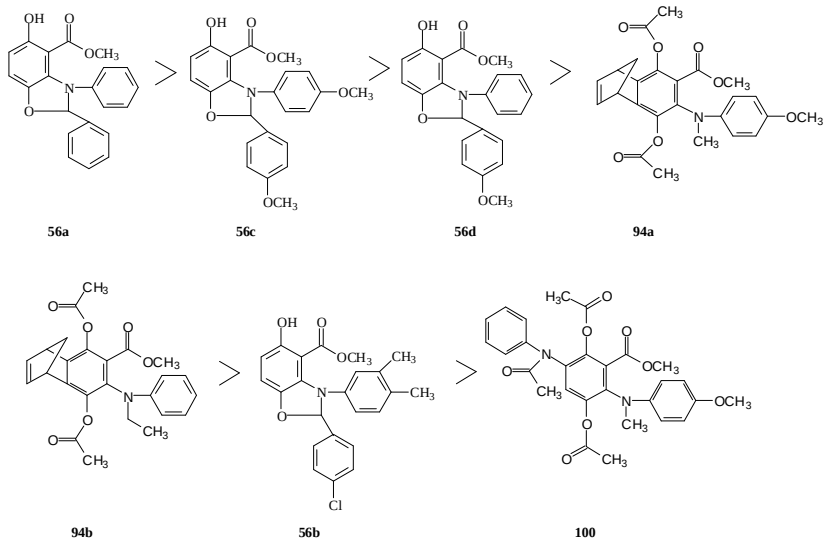
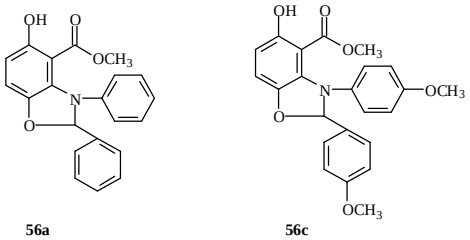


*Figure 28: Structure of cytotoxic compounds **56a**, **56c**, **56d**, **94a**, **94b**, **56b, 100**.*

In an attempt to look more deeply into the compound-induced morphological effects by visualization of intracellular structures and microscopy, PtK_2 cells were subject to compound treatment followed by staining of nuclei and the endoplasmatic reticulum (ER). Six compounds (**94a, 100, 94c, 56a, 56b, 56c**) showed interesting effects in the morphology of the cells. **94a, 100, 56a,** and **56b** caused severe changes of the inner structure of the cells. The cells showed large vacuoles, deformations of the nuclei, and vesicles in the ER. In some cases the ER was almost completely dissolved. Especially with **56a**, but also with **56b**, the entire

ER network was broken down to vesicles only after 4 hours. **100** and **56c** did not induce such severe damage of the ER system, only wider meshes in the ER network could be observed.
With **94c** a patch dense of ER is observed beside the nucleus, while the rest of the ER remains unaltered. Immunoflurescence staining with antidodies against gamma-tubulin showed that this patches are not connected to the centrosomes, their locations being independent of this organelles. The observed effect on the ER structure resembles strongly the action of several natural products such as corallidictyals, siphonodictyols and myricetin (flavonol).
The induced changes in the morphology of the cells differ in strength and in detail for each compound. But overall it is difficult to decide whether the phenotypes are due different mode of action. The different phenomena seem to overlap.

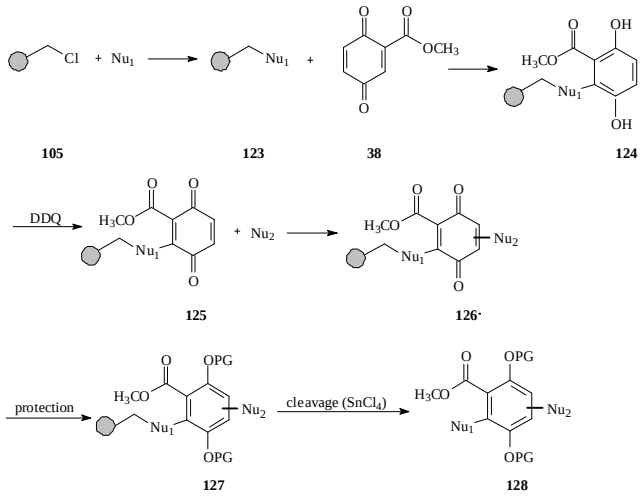
The benzoquinone derivatives were also tested for bactericidal, fungal and antibiotic activity in microbiological assays. None of the benzoquinone derivatives tested show antibacterial or fungal activity. Modest antibiotic activity against *Staphylococcus aureus, Mycobacterium phlei* and *Pseudomonas aeruginosa* was observed for two compounds **56a, 56c** (*Figure 26*).



56a **56c**

*Figure 26: Structure of active compounds **56a** and **56c**.*

After the very promising results with the first model compounds, application of the Michael addition concept to a multistep solid-phase synthesis was attempted. After establishing the synthetic method on solid phase (*Scheme 39*), a few quinone derivatives were synthesized in a multistep way by using Michael addition as key step.



Scheme 38: Implementation of Michael addition concept to solid phase.

The immobilization of 2-methoxycarbonyl-1,4-benzoquione **(38)** scaffold was achieved via Michael addition to the previously functionalized Merifield resin **(123)** with formation of the intermediate **(125)**. After the addition of the second nucleophile, the resulted intermediate **(126)** was protected by acetylation or methylation. Cleavage with $SnCl_4$ furnished the benzoquinone derivative **(128)**. An exception from the general strategy must be mentioned in the case of formation of benzoxazoline as final product, where the reaction cycle is finish after the first addition (*see Chapter 5.3 and 5.4*).

From all these above reactions it can be concluded that the Michael addition approach has been successfully applied in a multistep solid-phase synthesis and the first attempts in order to synthesize the benzoquinone library on solid phase have been done.

8 Material and Methods

8.1 Synthetic materials and methods

8.1.1 General

Nuclear Magnetic Resonance Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on the following instruments

Bruker DPX	300 MHz, ^{1}H NMR, 75 MHz, ^{13}C NMR
Bruker ARX	400 MHz, ^{1}H NMR, 100 MHz, ^{13}C NMR
Bruker Avance DMX	600 MHz, ^{1}H NMR, 151 MHz, ^{13}C NMR

with tetramethylsilane as the internal reference. The chemical shifts are provided in ppm and the coupling constants in Hz. The following abbreviations for multiplicities are used: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quadruplet; m, multiplet.

Mass Spectrometry

The high and low-resolution EI-mass spectra were recorded on a Finnigan MAT MS 95 (Bremen/Germany). Positive ion electrospray ionisation mass spectra (ESI-MS) were recorded on a micromass QTOF 2 mass spectrometer (Micromass, Manchester, UK). The isotopic composition of the sample was determinate in the accurate mass mode using sacharose ([M + Na] = 365.106 Da) as an internal reference compound.

Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) plates were obtained from Merck (Silica gel 60, F_{254}). The TLCs were visualized by UV light (λ = 254 nm, 366 nm) or by staining with iod. The solvent system and R_f values are noted for the synthesized compounds.

Preparative Layer Chromatography (PLC)

Preparative layer chromatography plates were obtained from Merck (silica gel 60, F_{254}, 2 mm, 1 mm and 0.5 mm).

Flash Chromatography

Flash column chromatography[83] was performed using flash silica gel (Merck, Darmstad, 40-64 μM) with pressure ranging from 0.5-1.0 bar. A 70-100 fold excess of silica gel to the crude product was used.

Melting Point

The melting points were measured with a Büchi 510 melting point apparatus.

Chemicals

Chemicals were obtained from the following suppliers and used without further purification: Aldrich, Fluka, Merck, Lancaster, Novabiochem, and Baker. Dry solvents were purchased from Fluka.

General working procedures (GWP)

General working procedure 1 (GWP1): Reduction of disubstituited benzoquinones

To a solution of disubstituited benzoquinone (1 equiv.) in DCM was added a saturated solution of $Na_2S_2O_4/H_2O$ (10 ml). The mixture was stirred at room temperature until the colour of the quinone had disappeared. The organic layer was evaporated under reduced pressure. The product was used in the next step without further purification.

General working procedure 2 (GWP2): Alkylation of disubstituited hydroquinones

To a solution of disubstituited hydroquinone (1 equiv.) in acetone (9-17 ml/mmol), was added MeI (11-12 equiv.) and K_2CO_3 (3 equiv.). The reaction mixture was heated under reflux overnight. Afterwards the solvent was evaporated and the crude product was purified to give the alkylated product.

General working procedure 3 (GWP3): Addition of cyclopentadiene to monosubstituted benzoquinones

To a solution of monosubstituted benzoquinone (1 equiv.) in ether (1.75-4.76 ml/mmol) at 0-5 °C was added a solution of freshly cracked cyclopentadiene (2.52-6.85 equiv.). The reaction mixture was stirred at room temperature for 3-4 h. After completion of the reaction, the solvent was evaporated under vacuum. Preparative layer chromatography (DCM/MeOH, 9:1) gave the product.

General working procedure 4 (GWP4): Acetylation of 7-substituted-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester

To a solution of 7-substituted-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester (1 equiv.) in pyridine (1.21-1.35 ml/mmol) was added acetic anhydride (4-7 equiv.). The homogeneous reaction mixture was allowed to stand at room temperature for seven days. After the reaction time has passed, the solvent was evaporated under reduced pressure and the resulting mixture separated by preparative layer chromatography under conditions described in the individual experimental chapters.

General working procedure 5 (GWP5): Addition of alcohols to 2-methoxycarbonyl-1,4-benzoquinone[57]

A mixture of 2-methoxycarbonyl-1,4-benzoquinone (2 equiv.), alcohol (1 equiv.) and $MgCl_2$ (1 equiv.) in toluene (6.5 ml/mmol) was stirred overnight at room temperature under N_2 atmosphere. Work up was carried out as described for the individual experiments.

General working procedure 6 (GWP6): Addition of thiophenol to monosubstituited benzoquinones

To a solution of of monosubstituited benzoquinone (1 equiv.) in toluene (3.33 ml/mmol) was added thiophenol (1.2 equiv.) and a solution of 2-methoxypyridine (3.16 equiv.) in toluene (0.53 ml/mmol). The reaction mixture was stirred overnight at room temperature. At the end of this time the solvent was evaporated and the residue purified by preparative layer chromatography under conditions described in the individual experimental chapters.

General working procedure 7 (GWP7): Synthesis of benzoxazolines

To a solution of 2-methoxycarbonyl-1,4-benzoquinone (1 equiv.) in toluene (1.66 ml/mmol) was added a solution of secondary amine (2 equiv.) in toluene (0.83 ml/mmol) and $MgSO_4$ (0.84 equiv.). The reaction mixture was stirred at room temperature overnight. Afterwards the solvent was evaporated and the residue purified by preparative layer chromatography.

General working procedure 8 (GWP8): Cleavage with $SnCl_4$[78]

To the swollen Merrifield resin (1equiv.) in dry DCM, was added under N_2, $SnCl_4$ (10 equiv.). The mixture was stirred overnight at room temperature; the excess of $SnCl_4$ was removed by extraction with water. Organic layer was evaporated under reduced pressure and purified if was necessary.

General working procedure 9 (GWP9): Resin loading with Boc-amino acid[69]

A solution of caesium salt of Boc-amino acid (4.8 equiv., 0.60 mmol, 0.19 g) and KI (0.4 equiv., 0.05 mmol, 8.30 mg) in 0.80 ml DMF was added to the swollen chloromethylpolystyrene resin **77** (0.10 g, loading 1.25 mmol/g, 0.125 mmol, 1 equiv.) which was previous washed with dry DMF (3x) before. After heating at 50 °C overnight, the solution was filtered off; the resin was washed with DMF, DMF/water, DCM, MeOH, followed by Boc deprotection with 50 % TFA/DCM for 15 min and washing with DMF, DCM, to provide the corresponding amino acid Merrifield resin.

Preparation of caesium salt of Boc-amino acid:

To a solution of Boc-amino acid (1mmol/2ml) in EtOH was added water (0.5 ml/mmol). Then the pH was adjusted to 7 with 2M aqueous solution of Cs_2CO_3. Afterwards the solvent was evaporated under reduced pressure and the residue was redissolved in dioxane and evaporated followed by drying.

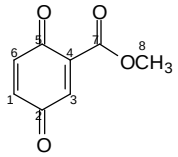
General working procedure 10 (GWP10): Loading determination[77]

Fmoc-amino acid Merrifield resin (1.00 mg) was added in 3 ml 20 % piperidine/DMF followed by shaking for 15 min. For reference a second cuvette with 1 ml 20 % piperidine in DMF was prepared. Spectrophotometric analysis was carried out at ? = 290 nm (e = 5800 M^{-1} cm^{-1}), where the resultant fluvene-piperidine adduct has UV absorption maxima.

Loading (mmol/g) = Absorption sample / mg of sample x 1.65

8.1.2 Compounds from Chapter 3.2

2-Methoxycarbonyl-1,4-benzoquinone



39

To a mixture of methyl-2,5-dihydroxybenzoate **38** (1.00 g, 5.95 mmol), K_2CO_3 (1.00 g, 3.28 mmol) in 50 ml toluene was added Ag_2O (3.00 g, 13.00 mmol). The reaction mixture was stirred at 50 °C for 30 minutes. After that, the solid was filtered off and the organic phase evaporated under reduced pressure to give the title compound in 90 % yield as orange solid, which was further use without any purification.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.10 (d, ^{4}J (H-3, H-1) = 2.6 Hz, 1H, H-3), 6.93 (d, ^{3}J (H-1, H-6) = 8.0 Hz, ^{4}J (H-1, H-3) = 2.6 Hz, 1H, H-1), 6.91 (^{3}J (H-6, H-1) = 8.0 Hz, 1H, H-6), 3.92 (s, 3H, H-8).

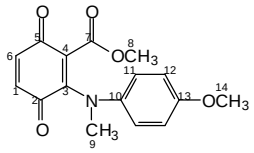
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 186.79 (C-5), 182.95 (C-2), 163.16 (C-7), 137.19 (C-4), 136.96 (C-1*), 136.53 (C-3), 136.17 (C-6*), 53.11 (C-8).

*These assignments are interchangeable.

HR-EI-MS: *m/z* (%) = 166.0267 (M^+, calcd 166.0266 for $C_8H_6O_4$).

The spectroscopic data is in agreement with the assigned structure and those reported by Brunner K.[52]

3-(N-Methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone



50a

To a solution of 2-methoxycarbonyl-1,4-benzoquinone **39** (0.60 g, 3.61 mmol) in 6 ml toluene was added N-methyl-4-anisidine (0.37 g, 2.73 mmol) and $MgSO_4$ (1.20 g, 10.00 mmol). After stirring at room temperature 3 h, Ag_2O (2.40 g, 10.35 mmol) was added. Afterwards the reaction mixture was stirred at room temperature overnight. Purification was made by flash chromatography on silica gel (eluent: toluene/EtOAc, 9:1), followed by crystallization from acetone/PEE (1:1), to afford **50a** in a 67 % yield as violet crystals.

R_f = 0.05 (acetone/PEE = 1:1)

m.p. = 113-114 °C.

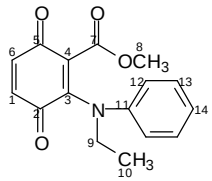
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.04 (d, ^{3}J (H-12, H-11) = 9.0 Hz, 2H, H-12), 6.85 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 6.66 (d, ^{3}J (H-6, H-1) = 10.2 Hz, 1H, H-6), 6.55 (d, ^{3}J (H-1, H-6) = 10.2 Hz, 1H, H-1), 3.79 (s, 3H, H-14), 3.56 (s, 3H, H-8), 3.42 (s, 3H, H-9).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 184.75 (C-5), 182.94 (C-2), 165.80 (C-7), 157.97 (C-13), 148.05 (C-3), 139.76 (C-10), 137.22 (C-6), 134.43 (C-1), 126.31 (C-11), 114.57 (C-12), 117.81 (C-4), 55.55 (C-14), 52.19 (C-8), 43.89 (C-9).

MS-ESI: *m/z* (%) = 324 [M^+ + Na], 302 [M^+ + H].

The spectroscopic data is in agreement with the assigned structure and those reported by Müller *et al*.[51]

3-(Ethyl-phenyl-amino)-2-methoxycarbonyl-1,4-benzoquinone



50b

To a solution of 2-methoxycarbonyl-1,4-benzoquinone **39** (0.60 g, 3.61 mmol) in 6 ml toluene was added N-ethylaniline (0.02 g, 0.18 mmol) and $MgSO_4$ (1.20 g, 9.96 mmol). After 3 h stirring at room temperature, Ag_2O (0.10 g, 0.43 mmol) was added. The reaction mixture was stirred at room temperature overnight. Purification was made by flash chromatography on silica gel (eluent: toluene/EtOAc, 9:1) to provide the title compound in 50 % yield as violet oil.

R_f = 0.16 (toluene/EtOAc = 9:1)

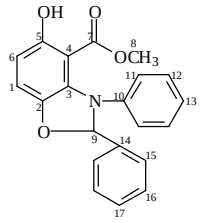
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.33 (t, ^{3}J (H-12, H-13) = 7.6 Hz, 2H, H-12), 7.18 (t, 1H, H-14), 7.08 (d, ^{3}J (H-13, H-12) = 7.6 Hz, 2H, H-13), 6.67 (d, ^{3}J(H-1, H-6) = 12.7 Hz, ^{3}J (H-6, H-1) = 12.7 Hz, 2H, H-1, H-6), 3.85 (q, 2H, H-9), 3.30 (s, 3H, H-8), 1.21 (t, 3H, H-10).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 185.28 (C-2), 183.05 (C-5), 165.16 (C-7), 148.09 (C-3), 144.03 (C-11), 137.11 (C-6), 134.78 (C-1), 129.22 (C-13), 125.12 (C-12), 124.19 (C-14), 120.07 (C-4), 49.36 (C-9), 13.74 (C-10).

ESI-MS: *m/z* (%) = 593 [$2M^+$ + Na], 308 [M^+ + Na], 286 [M^+ + H].

HR-EI-MS: *m/z* (%) = 285.1004 (M^+, calcd 285.1001 for $C_{16}H_{15}NO_4$).

5-Hydroxy-2,3-diphenyl-2,3-dihydro-benzooxazole-4-carboxylic acid methyl ester



56a

Compound **56a** was synthesized according to *GWP7* starting from 2-methoxycarbonyl-1,4-benzoquinone **39** (0.12 g, 0.72 mmol) and N-phenyl benzyl amine (0.07 g, 0.36 mmol). The crude product was purified by preparative layer chromatography (eluent: toluene/EtOAc, 9:1) to provide the title compound in 15 % yield as yellow solid.

R_f = 0.68 (toluene/EtOAc = 9:1)

m.p. = 120-121 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.16 (s, 1H, OH), 7.58 (m, ^{3}J (H-15, H-16) = 5.1 Hz, 2H, H-15), 7.41 (m, ^{4}J (H-15, H-17) = 2.1 Hz, ^{3}J (H-16, H-15) = 5.1 Hz, 3H, H-17, H-16), 7.25 (d, ^{3}J (H-12, H-11) = 8.3 Hz, 2H, H-12), 7.08 (t, ^{3}J (H-13, H-12) = 8.5 Hz, 1H, H-13), 6.92 (d, 2H, H-11), 6.90 (d, ^{3}J (H-1, H-6) = 8.5 Hz, 1H, H-1), 6.58 (s, 1H, H-9), 6.50 (d, ^{3}J (H-6, H-1) = 8.48 Hz, 1H, H-6), 3.31 (s, 3H, H-8).

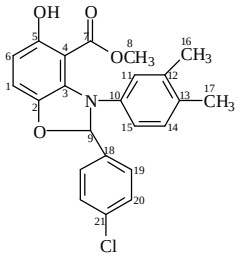
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 169.16 (C-7), 155.43 (C-5), 147.13 (C-10), 144.67 (C-2), 139.40 (C-14), 135.26 (C-3), 129.55 (C-17), 129.15 (C-12), 128.82 (C-16), 126.84 (C-15), 124.40 (C-13), 120.39 (C-11), 114.78 (C-1), 109.07 (C-6), 102.24 (C-9), 102.07 (C-4), 51.28 (C-8).

ESI-MS: *m/z* (%) = 371 [M^+ + H + Na], 370 [M^+ + Na], 348 [M^+ + H].

EI-MS (70eV): *m/z* (%) = 347 (55) [M^+], 315 (100) [M^+, -OCH_3, -H], 270 (11) [M^+ -C_6H_5], 238 (38) [M^+ -C_6H_5, -OCH_3, -H].

HR-EI-MS: *m/z* (%) = 347.1163 (M^+, calcd 347.1157 for $C_{21}H_{17}NO_4$).

2-(4-Chloro-phenyl)-3-(3,4-dimethyl-phenyl)-5-hydroxy-2,3-dihydro-benzooxazole-4-carboxylic acid methyl ester



56b

Compound **56b** was synthesized according to *GWP7* starting from 2-methoxycarbonyl-1,4-benzoquinone **39** (0.10 g, 0.60 mmol) and 4-chloro-N-(3,4-dimethylphenyl) benzyl amine (0.07 g, 0.30 mmol). The crude product was purified by preparative layer chromatography (eluent: toluene/EtOAc, 9:1) to provide the title compound in 8 % yield as yellow solid.

R_f = 0.52 (toluene/EtOAc = 9:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.12 (s, 1H, OH), 7.50 (d, ^{3}J (H-19, H-20) = 8.4 Hz, 2H, H-19), 7.40 (d, ^{3}J (H-20, H-19) = 8.4 Hz, 2H, H-20), 6.96 (d, ^{3}J (H-14, H-15) = 8.1 Hz, 1H, H-14), 6.85 (d, ^{3}J (H-1, H-6) = 8.4 Hz, 1H, H-1), 6.65 (dd, ^{3}J (H-11, H-15) = 2.2 Hz, 1H, H-11), 6.62 (d, ^{3}J (H-15, H-14) = 8.1 Hz, 1H, H-15), 6.46 (s, 1H, H-9), 6.45 (d, ^{3}J (H-6, H-1) = 8.4 Hz, 1H, H-6), 3.31 (s, 3H, H-8), 2.20 (s, 3H, H-17), 2.16 (s, 3H, H-16).

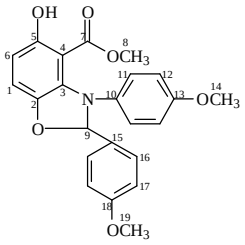
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 169.12 (C-7), 155.46 (C-5), 144.95 (C-10), 144.48 (C-2), 138.15 (C-18), 137.62 (C-12), 135.82 (C-3), 135.41 (C-21), 133.11 (C-13), 130.22 (C-14), 128.98 (C-20), 128.38 (C-19), 121.93 (C-11), 118.34 (C-15), 114.59 (C-1), 108.88 (C-6), 102.05 (C-4), 101.77 (C-9), 51.29 (C-8), 19.82 (C-16), 19.17 (C-17).

ESI-MS: *m/z* (%) = 432 [M^+ + Na], 410 [M^+ + H].

EI-MS (70eV): *m/z* (%) = 409 (88) [M^+], 377 (100) [M^+ -OCH_3, -H], 348 (36) [M^+ -OCH_3, -$2CH_3$], 266 (76) [M^+ -OCH_3, -C_6H_4Cl, -H].

HR-EI-MS: *m/z* (%) = 409.1074 (M^+, calcd 409.1080 for $C_{23}H_{20}ClNO_4$).

5-Hydroxy-2,3-di-*p*-tolyl-2,3-dihydro-benzooxazole-4-carboxylic acid methyl ester



56c

Compound **56c** was synthesized according to *GWP7* starting from 2-methoxycarbonyl-1,4-benzoquinone **39** (0.10 g, 0.60 mmol) and 4-methoxy-N-(4-methoxyphenyl) benzyl amine (0.07 g, 0.39 mmol). The crude product was purified by preparative layer chromatography (eluent: toluene/EtOAc, 9:1) to provide the title compound in 13 % yield as yellow oil.

R_f = 0.52 (toluene/EtOAc = 9:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.10 (s, 1H, OH), 7.51 (d, ^{3}J (H-16, H-17) = 8.7 Hz, 2H, H-16), 6.94 (d, ^{3}J (H-17, H-16) = 8.7 Hz, 2H, H-17), 6.85 (d, ^{3}J (H-1, H-6) = 10.4 Hz, 1H, H-1), 6.82 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 6.77 (d, ^{3}J (H-12, H-11) = 9.0 Hz, 2H, H-12), 6.42 (d, ^{3}J (H-6, H-1) = 10.4 Hz, 1H, H-6), 6.40 (s, 1H, H-9), 3.82 (s, 3H, H-14*), 3.75 (s, 3H, H-19*), 3.28 (s, 3H, H-8).

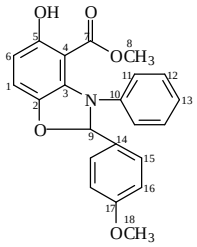
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 169.17 (C-7), 160.64 (C-18), 156.94 (C-13), 155.21 (C-5), 144.53 (C-2), 140.16 (C-3), 137.04 (C-10), 131.40 (C-14), 128.56 (C-16), 123.18 (C-17), 114.37, 114.09 (C-12*, C-11*, C-1*), 107.80 (C-6), 102.86 (C-9), 100.98 (C-4), 55.52 (C-14), 55.38 (C-19), 51.31 (C-8).

* These assignments are interchangeable.

ESI-MS: *m/z* (%) = 837 [M^+ + Na], 446 [M^+ + K], 430 [M^+ + Na], 408 [M^+ + H].

HR-EI-MS: *m/z* (%) = 407.1357 (M^+, calcd 407.1368 for $C_{23}H_{21}NO_6$).

5-Hydroxy-2-(4-methoxy-phenyl)-3-phenyl-2,3-dihydro-benzooxazole-4-carboxylic acid methyl ester



56d

Benzooxazole **56d** was synthesized according to *GWP7* starting from 2-methoxycarbonyl-1,4-benzoquinone **39** (0.12 g, 0.72 mmol) and 4-methoxy-N-phenyl benzyl amine (0.07 g, 0.36 mmol). The crude product was purified by preparative layer chromatography (eluent: toluene/EtOAc, 9:1) to provide the title compound in 6 % yield as yellow solid.

R_f = 0.70 (toluene/EtOAc = 9:1)

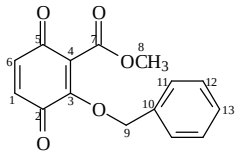
m.p. = 107-108 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.12 (s, 1H, OH), 7.50 (d, ^{3}J (H-15, H-16) = 8.5 Hz, 2H, H-15), 7.23 (dd, ^{3}J (H-12, H-11) = 7.3 Hz, 2H, H-12), 7.06 (t, 1H, H-13), 6.95 (d, ^{3}J (H-16, H-15) = 8.5 Hz, 2H, H-16), 6.92 (d, ^{3}J (H-11, H-12) = 7.3 Hz, 2H, H-11), 6.88 (d, ^{3}J (H-1, H-6) = 8.7 Hz, 1H, H-1), 6.52 (s, 1H, H-9), 6.47 (d, ^{3}J (H-6, H-1) = 8.7 Hz, 1H, H-6), 3.82 (s, 3H, H-18), 3.27 (s, 3H, H-8).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 169.05 (C-7), 160.52 (C-17), 154.83 (C-5), 146.79 (C-10), 144.56 (C-2), 135.45 (C-3), 131.48 (C-14), 129.05 (C-12), 128.22 (C-15), 124.38 (C-13), 120.44 (C-11), 114.46 (C-1), 114.08 (C-16), 108.47 (C-6), 102.08 (C-9), 101.65 (C-4), 55.28 (C-18), 51.15 (C-8).

ESI-MS: *m/z* (%) = 401 [M^+ + Na], 378 [M^+ + H].

2-Methoxycarbonyl-3-benzyloxy-1,4-benzoquinone



69a

Benzoquinone **69a** was synthesized according to *GWP5* starting from 2-methoxycarbonyl-1,4-benzoquinone **39** (0.50 g, 3.00 mmol) and benzyl alcohol (0.15 g, 1.50 mmol) . The crude product was purified by flash chromatography in DCM to provide the title compound in 80 % yield as yellow oil.

R_f = 0.55 (DCM)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.35 (s, 5H, H-11, H-12, H-13), 6.82 (s, 2H, H-1, H-6), 5.39 (s, 2H, H-9), 3.82 (s, 3H, H-8).

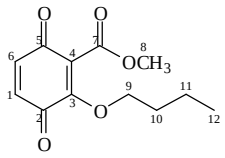
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 184.15 (C-2), 182.58 (C-5), 163.39 (C-7), 153.60 (C-3), 135.26 (C-1), 135.75 (C-10), 134. 77 (C-6), 128.60 (C-12), 128.70 (C-13), 127.96 (C-11), 122.24 (C-4), 74.60 (C-9), 52.71 (C-8).

ESI-MS: *m/z* (%) = 567 [$2M^+$ + Na], 295 [M^+ + Na], 273 [M^+ + H].

HR-EI-MS: *m/z* (%) = 272.0690 (M^+, calcd 272.0685 for $C_{15}H_{12}O_5$).

The spectroscopic data is in agreement with the assigned structure and those reported by Hormi *et al*.[57]

2-Methoxycarbonyl-3-butoxy-1,4-benzoquinone



69b

Monosubstituted benzoquinone **69b** was synthesized according to *GWP5* starting from 2-methoxycarbonyl-1,4-benzoquinone **39** (3.91 g, 23.50 mmol) and 1-butanol (0.89 ml, 12 mmol). The crude product was purified by chromatography (eluent: DCM) to provide the title compound in 16 % yield as yellow oil.

R_f = 0.64 (DCM)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.70 (s, 2H, H-1, H-6), 4.30 (t, 2H, H-9), 3.88 (s, 3H, H-8), 1.70 (m, 2H, H-10), 1.42 (m, 2H, H-11), 0.90 (t, 3H, H-12).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 184.50 (C-2), 182.34 (C-5), 164.04 (C-7), 153.83 (C-3), 122.48 (C-1), 120.17 (C-4), 113.41 (C-6), 72.77 (C-9), 52.83 (C-8), 31.71 (C-10), 18.75 (C-11), 13.59 (C-4).

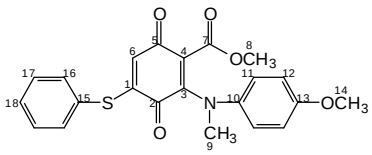
ESI-MS: *m/z* (%) = 262 [M^+ + H + Na], 261 [M^+ + Na], 239 [M^+ + H].

HR-EI-MS: *m/z* (%) = 238.0825 (M^+, calcd 238.0841 for $C_{12}H_{14}O_5$).

The spectroscopic data is in agreement with the assigned structure and those reported by Hormi *et al*.[57]

8.1.3 Compounds from Chapter 3.3

5-Phenylsulfanyl-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone



74

To a solution of 3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **50a** (0.15 g, 0.49 mmol) in 1.63 ml toluene was added thiophenol **73** (0.06 ml, 0.60 mmol), a solution of (0.16 ml, 1.57 mmol) of 2-methoxypyridine in 0.84 ml toluene, Ag_2O (0.21 g, 1.23 mmol) and $MgSO_4$ (0.16 g, 1.37 mmol). After being stirred at room temperature overnight, the reaction solution was concentrated and purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: toluene/EtOAc, 9:1) to afford **74** in 55 % as blue solid.

R_f = 0.20 (toluene/EtOAc = 9:1)

m.p. = 145-146 °C.

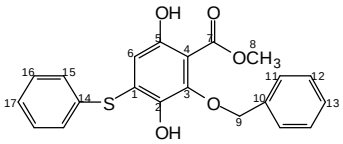
^{1}H-NMR (300 MHz, CD_3CN): δ = 7.56 (m, 5H, H-16, H-17, H-18), 7.17 (d, ^{3}J(H-11, H-12)= 8.7 Hz, 2H, H-11), 6.85 (d, ^{3}J (H-12, H-11) = 8.7 Hz, 2H, H-12), 5.62 (s, 1H, H-6), 3.81 (s, 3H, H-14), 3.52 (s, 3H, H-8), 3.35 (s, 3H, H-9).

^{13}C-NMR (75 MHz, CD_3CN): δ = 182.89 (C-5), 180.81 (C-2), 166.29 (C-7), 158.25 (C-13), 152.15 (C-1), 149.31 (C-3), 141.10 (C-10), 136.37 (C-16), 131.44 (C-15), 131.35 (C-17), 128.67 (C-18), 127.47 (C-6), 126.36 (C-11), 120.22 (C-4), 115.30 (C-12), 56.18 (C-14), 52.62 (C-8), 43.62 (C-9).

MS-ESI: *m/z* (%) = 841 [$2M^+$ + Na], 432 [M^+ + Na], 410 [M^+].

HR-ESI-MS: *m/z* (%) = 410.1060 (M^+ + H, calcd 410.1062 for $C_{22}H_{20}NO_5S$).

2-Benzyloxy-3,6-dihydroxy-4-phenyl sulfanyl-benzoic acid methyl ester



77a

Disubstituted hydroquinone **77a** was synthesized according to *GWP6* starting from intermediate **69a** (0.08 g, 0.30 mmol) and thiophenol (37 µl, 0.36 ml). The crude product was purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: toluene/EtOAc, 9:1) to provide the title compound in 16 % yield.

R_f = 0.85 (toluene/EtOAc = 20:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.67 (s, 1H, HO-5), 7.37-7.42 (m, 10H, H-11, H-12, H-13, H-15, H-16, H-17), 6.32 (s, 1H, H-6), 5.66 (s, 1H, HO-2), 4.95 (d, 2H, H-9), 3.95 (s, 3H, H-8).

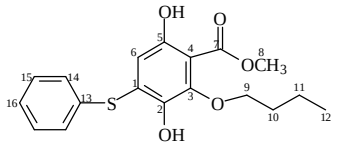
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 170.11 (C-7), 155.69 (C-5), 144.64 (C-3), 139.71 (C-2), 136.67 (C-10), 134.25 (C-1), 134.00 (C-15), 130.54 (C-14), 129.04, 129.81 (C-12, C-16), 128.85, 129.38 (C-17*, C-13*), 128.09 (C-11), 112.25 (C-6), 104.77 (C-4), 76.75 (C-9), 52.56 (C-8).

* These assignments are interchangeable.

EI-MS (70 eV): *m/z* (%) = 382 (16) [M^+], 351 (14) [M^+ -OCH_3], 291 (100) [M^+ -$CH_2C_6H_5$], 260 (22) [M^+ -$CH_2C_6H_5$, -OCH_3], 259 (95) [M^+ -$CH_2C_6H_5$, -OCH_3, -H], 91 (90) [$CH_2C_6H_5$].

HR-EI-MS: *m/z* (%) = 382.0877 (M^+, calcd 382.0874 for $C_{21}H_{18}O_5S$).

2-Butoxy-3,6-dihydroxy-4-phenyl sulfanyl benzoic acid methyl ester



77b

Hydroquinone **77b** was synthesized according to *GWP6* starting from intermediate **69b** (0.02 g, 0.08 mmol) and thiophenol (9.9 µl, 0.09 mmol). The crude product was purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: hexane/EtOAc, 9:1) to afford the title compound in 29 % yield as yellow solid.

R_f = 0.56 (hexane/EtOAc = 8:2)

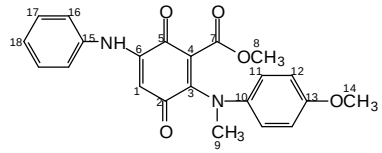
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.62 (s, 1H, HO-5), 7.51-7.35 (m, 5H, H-14, H-15, H-16), 6.22 (s, 1H, H-6), 5.46 (s, 1H, HO-2), 3.99 (s, 3H, H-8), 3.87 (t, 2H, H-9), 1.80 (m, 2H, H-10), 1.49 (m, 2H, H-11), 0.99 (t, 3H, H-12).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.60 (C-7), 155.62 (C-5), 144.73 (C-3), 139.21 (C-2), 136.00 (C-1), 134.42 (C-14), 130.09 (C-13), 129.83 (C-15), 129.20 (C-16), 111.27 (C-6), 104.11 (C-4), 75.49 (C-9), 52.55 (C-8), 32.23 (C-10), 19.27 (C-11), 13.95 (C-12).

ESI-MS: *m/z* (%) = 371 [M^+ + Na], 349 [M^+ + H].

HR-ESI-MS: *m/z* (%) = 349.1120 (M^+, calcd 349.1110 for $C_{18}H_{20}O_5S$).

6-Phenyl amino-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone



80

To a solution of **50a** (0.20 g, 0.66 mmol) in 2 ml toluene was added aniline (0.12 ml, 1.32 mmol). After being stirred at room temperature overnight, the reaction solution was concentrated to dryness and the residue purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: CH_2Cl_2/EtOAc, 9:1) to afford **80** in 60 % yield as brown solid.

R_f = 0.81 (DCM/EtOAc = 9:1)

m.p. =147-148 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.77 (s, 1H, NH), 7.42 (t, ^{3}J (H-17, H-16) = 8.0 Hz, 3H, H-17), 7.20 (d, ^{3}J (H-16, H-17) = 8.6 Hz, 2H, H-16), 7.12 (t, 1H, H-18), 7.12 (d, ^{3}J (H-12, H-11) = 9.0, 2H, H-12), 6.98 (d, ^{3}J (H-11, H-12) = 9.0, 2H, H-11), 5.98 (s, 1H, H-1), 3.79 (s, 3H, H-14), 3.56 (s, 3H, H-8), 3.44 (s, 3H, H-9).

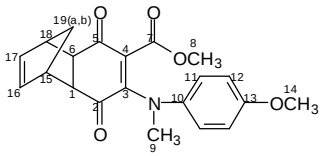
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 182.10 (C-5), 177.7 (C-2), 166.18 (C-7), 158.30 (C-13), 151.62 (C-3), 144.0 (C-15), 139.80 (C-10), 137.41 (C-6), 129.60 (C-16), 127.0 (C-17), 126.40 (C-11), 125.61 (C-18), 123.80 (C-4), 114.40 (C-12), 98.90 (C-1), 55.53 (C-14), 51.90 (C-8), 45.50 (C-9).

EI-MS (70eV): *m/z* (%) = 392 (100) [M^+], 361 (37) [M^+ -OCH_3].

MS-ESI: *m/z* (%) = 807 [2(M^+) + Na], 415 [M^+ + Na], 393 [M^+ + H].

HR-EI-MS: *m/z* (%) = 393.1460 (M^+ + H, calcd 393.1450 for $C_{22}H_{21}N_2O_5$).

7-(N-methyl-4`-anisidino)-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-carboxy-lic-acid methyl ester



90a

Intermediate **90a** was synthesized according to *GWP3* starting from 3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **50a** (0.10 g, 0.33 mmol) and cyclopentadiene (0.05 g, 0.84 mmol). The crude product was purified by flash chromatography (eluent: DCM/MeOH, 9:1) to provide the title compound in 96 % yield as orange solid.

R_f = 0.39 (DCM/MeOH = 9:1)

m.p. = 55-56 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.02 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 6.84 (d, ^{3}J(H-12, H-11) = 9.1 Hz, 2H, H-12), 6.21 (dd, ^{3}J (H-16, H-17, H-15) = 5.7 Hz, 1H, H-16), 6.14 (dd, ^{3}J (H-17, H-16, H-18) = 5.70 Hz, 1H, H-17), 3.80 (s, 3H, H-14), 3.53 (m, 1H, H-15), 3.43 (s, 3H, H-8), 3.35 (m, 1H, H-18), 3.27 (s, 3H, H-9), 3.31 (dd, ^{3}J (H-1, H-15) = 9.2 Hz, ^{3}J (H-1, H-6) = 3.8 Hz, 1H, H-1), 3.20 (dd, ^{3}J (H-6, H-18) = 9.2 Hz, ^{3}J (H-6, H-1) = 3.8 Hz, 1H, H-6), 1.52 (t, 1H, H-19a), 1.31 (t, 1H, H-19b).

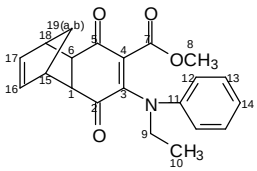
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 196.82 (C-2*), 193.24 (C-5*), 166.50 (C-7), 158.27 (C-13), 154.07 (C-3), 139.10 (C-10), 136.57 (C-16**), 135.31 (C-17**), 126.86 (C-11), 119.60 (C-4), 114.49 (C-12), 55.54 (C-14), 51.99 (C-8), 50.34 (C-6), 49.77 (C-1), 48.60 (C-19), 47.15, 47.54 (C-15, C-18), 43.79 (C-9).

*, ** These assignments are interchangeable.

EI-MS (70 eV): *m/z* (%) = 367 (67) [M^+], 301 (100) [M^+ -C_5H_6].

HR-EI-MS: *m/z* (%) = 367.1395 (M^+, calcd 367.1419 for $C_{21}H_{21}NO_5$).

7-(Ethyl-phenyl-amino)-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester



90b

Compound **90b** was synthesized according to *GWP3* starting from intermediate **50b** (0.06 g, 0.21 mmol) and cyclopentadiene (0.09 g, 1.44 mmol). The crude product was purified by preparative layer chromatography (eluent: DCM/MeOH, 9:1) to provide the title compound in 98 % yield as white oil.

R_f = 0.86 (DCM/MeOH = 9:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.32 (t, ^{3}J (H-12, H-13) = 8.5 Hz, 2H, H-12), 7.20 (t, ^{3}J (H-14, H-13) = 7.4 Hz, 1H, H-14), 7.05 (d, ^{3}J (H-13, H-12) = 8.5 Hz, 2H, H-13), 6.22-6.20 (m, ^{3}J (H-16, H-17, H-15) = 5.7 Hz, ^{3}J (H-17, H-16, H-18) = 5.7 Hz, H-16,H-17), 3.73 (q, 2H, H-9), 3.51 (m, 1H, H-15), 3.44 (m, 1H, H-18), 3.35 (m, ^{3}J (H-1, H-15) = 9.2 Hz, ^{3}J (H-1, H-6) = 3.8 Hz, ^{3}J(H-6, H-18) = 9.2 Hz, ^{3}J (H-6, H-1) = 3.8 Hz, 2H, H-1, H-6), 3.21 (s, 3H, H-8), 1.61 (t, 1H, H-19a), 1.40 (t, 1H, H-19b), 1.23 (t, 3H, H-10).

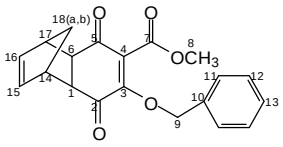
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 197.35 (C-2), 193.29 (C-5), 165.88 (C-7), 153.75 (C-3), 143.42 (C-11), 136.49 (C-16**), 135.19 (C-17**), 129.15 (C-12***), 126.61 (C-14), 126.32 (C-13***), 121.74 (C-4), 52.59 (C-8), 50.16 (C-6), 49.65 (C-1), 49.56 (C-9), 48.65 (C-19), 47.69 (C-15****), 47.43 (C-18****), 13.89 (C-10).

*, **, ***, **** These assignments are interchangeable.

ESI-MS: *m/z* (%) = 725 [$2M^+$ + Na], 374 [M^+ + Na], 352 [M^+ + H].

HR-EI-MS: *m/z* (%) = 351.1475 (M^+, calcd 351.1470 for $C_{21}H_{21}NO_4$).

7-Benzyloxy-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester



91

Intermediate **91** was synthesized according to *GWP3* starting from monosubstituited benzoquinone **77a** (0.15 g, 0.57 mmol) and cyclopentadiene (0.09 g, 1.44 mmol). The crude product was purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: DCM/MeOH, 9:1) to provide the title compound in 95 % yield as yellow oil.

R_f = 0.56 (DCM/MeOH = 9:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.21-7.40 (m, 5H, H-11, H-12, H-13), 6.11 (m, ^{3}J (H-15, H-16, H-14) = 5.1 Hz, 1H, H-15), 5.66 (m, ^{3}J (H-16, H-17, H-15) = 5.1 Hz, 1H, H-16), 5.45-5.28 (s, 2H, H-9), 3.76 (s, 3H, H-8), 3.49-3.30 (m, 2H, H-14, H-17), 3.20 (q, 1H, H-6), 3.12 (q, 1H, H-1), 1.49 (d, 1H, H-18a), 1.25 (t, 1H, H-18b).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 195.19 (C-2*), 194.93 (C-5*), 163.88 (C-7), 158.38 (C-3), 135.73 (C-10), 135.51 (C-15**), 134.64 (C-16**), 129.64 (C-4), 128.51 (C-11), 128.46 (C-12***), 128.05 (C-13***), 73.92 (C-9), 52.41 (C-8), 49.36 (C-17****), 48.98 (C-18), 48.88 (C-14****), 48.76 (C-1), 48.67 (C-6).

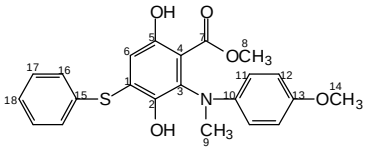
*, **, ***, **** These assignments are interchangeable.

ESI-MS: *m/z* (%) = 699 [$2M^+$ + Na], 362 [M^+ + H + Na], 361 [M^+ + Na].

HR-EI-MS: *m/z* (%) 338.1140 (M^+, calcd 367.1154 for $C_{20}H_{18}O_5$).

8.1.4 Compounds from Chapter 3.4

3,6-Dihydroxy-2-(N-methyl-4`-anisidino)-4-phenyl sulfanyl-benzoic acid methyl ester



93a

5-Phenyl-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **92** was reduced with saturated solution of $Na_2S_2O_4/H_2O$ to hydroquinone **93a** according to *GWP1*. A 100 % yield of the title compound was obtained as yellow solid.

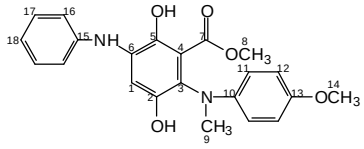
^{1}H-NMR (300 MHz, CD_3CN): δ = 10.65 (s, 1H, HO-5), 7.59 (t, 1H, H-18), 7.42 (d, ^{3}J (H-17, H-16) = 8.0 Hz, 2H, H-17), 7.46 (m, ^{3}J (H-16, H-17) = 8.0 Hz, 2H, H-16), 6.78 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 6.53 (d, ^{3}J (H-12, H-11) = 9.0 Hz, 2H, H-12), 6.42 (s,1H, HO-2), 6.31 (s, 1H, H-6), 3.73 (s, 3H, H-14), 3.56 (s, 3H, H-8), 3.21 (s, 3H, H-9).

^{13}C-NMR (75 MHz, CD_3CN): δ = 169.82 (C-7), 156.67 (C-5), 152.80 (C-13), 143.65 (C-2), 143.00 (C-10), 135.74 (C-1), 135.22 (C-16), 131.73 (C-3), 129.97 (C-17), 129.78 (C-18), 129.55 (C-15), 114.63 (C-12), 114.17 (C-11), 113.89 (C-6), 106.87 (C-4), 55.78 (C-14), 52.25 (C-8), 38.65 (C-9).

EI-MS (70 eV): *m/z* (%) = 411 (100) [M^+], 396 (8) [M^+ -CH_3], 302 (6) [M^+ -SC_6H_5].

HR-EI-MS: *m/z* (%) = 411.1090 (M^+, calcd 411.1140 for $C_{22}H_{21}NO_5S$).

2,5-Dihydroxy-6-(N-methyl-4`-anisidino)-3-phenyl amino benzoic acid methyl ester



93b

6-Phenyl amino-3-(N-methyl-4`-anisidino)-2-methoxycarbonyl-1,4-benzoquinone **92** (0.02 g, 0.05 mmol) was reduced with saturated solution of $Na_2S_2O_4/H_2O$ to the hydroquinone **93b** according to *GWP1*. A 100 % of the title compound was obtained as yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 11.15 (s, 1H, HO-5), 7.35 (t, ^{3}J (H-17, H-16) = 8.0 Hz, 2H, H-17), 7.22 (d, ^{3}J (H-16, H-17) = 8.0 Hz, 2H, H-16), 7.03 (t, 1H, H-18), 6.78 (d, ^{3}J (H-12, H-11) = 9.0 Hz, 2H, H-12), 6.51 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 6.41 (s, 1H, H-1), 6.10 (s, 1H, HO-2), 3.78 (s, 3H, H-14), 3.61 (s, 3H, H-8), 3.12 (s, 3H, H-9).

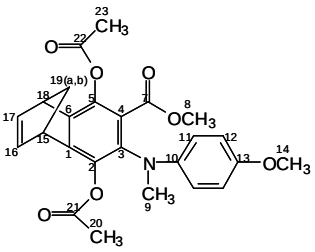
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.65 (C-7), 152.38 (C-13), 147.40 (C-2), 145.83 (C-5), 143.92 (C-10), 141.26 (C-15), 133.72 (C-6), 129.49 (C-16), 122.55 (C-18), 121.84 (C-3), 120.19 (C-17), 114.72 (C-12*), 113.72 (C-11*), 109.15 (C-4), 104.86 (C-1), 55.79 (C-14), 52.47 (C-8), 39.07 (C-9).

** These assignments are interchangeable.

ESI-MS: *m/z* (%) = 417 [M^+ + Na], 395 [M^+ + H].

HR-EI-MS: *m/z* (%) = 394.1429 (M^+, calcd 394.1528 for $C_{22}H_{22}N_2O_5$).

5,8-Diacetoxy-7-(N-methyl-4`-anisidino)-4-dihydro-1,4-methano-naphtalene-6-carboxy-lic acid methyl ester



94a

Compound **94a** was synthesized according to *GWP4* starting from 7-(N-methyl-4`-anisidino)-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester **93** (0.10 g, 0.28 mmol) and acetic anhydride (0.11 ml, 1.23 mmol). The crude product was purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: DCM/EtOAc, 9:1) to afford the title compound in 53 % yield as yellow solid.

R_f = 0.71 (DCM/EtOAc = 9:1)

m.p. = 73-74 °C

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.81 (dd, ^{3}J (H-17, H-16, H-18) = 5.0 Hz, 1H, H-17*), 6.77 (dd, ^{3}J (H-16, H-17, H-15) = 5.0 Hz 1H, H-16*), 6.71 (d, ^{3}J (H-12, H-11) = 9.0 Hz, 2H, H-12), 6.56 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 3.89 (s, 1H, H-18**), 3.85 (s, 1H, H-15**), 3.71 (s, 3H, H-14), 3.57 (s, 3H, H-8), 3.07 (s, 3H, H-9), 2.28 (m, 1H, H-19a***), 2.26 (s, 3H, H-20****), 2.21 (m, 1H, H-19b***), 1.96 (s, 3H, H-23****).

*, **, ***, **** These assignments are interchangeable.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.49 (C-21*), 168.33 (C-22*), 165.60 (C-7), 152.57 (C-13), 148.76 (C-1**), 143.38 (C-6**), 142.96 (C-10), 142.49 (C-16***), 142.14 (C-17***),

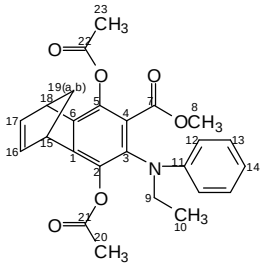
140.51 (C-5), 139.37 (C-2), 137.42 (C-3), 124.54 (C-4), 115.21 (C-11****), 114.38 (C-12****), 67.87 (C-19), 55.75 (C-14), 52.18 (C-8), 48.64 (C-15*****), 48.21 (C-18*****), 39.53 (C-9), 20.58 (C-20******), 20.29 (C-23******).

*, **, ***, ****, *****, ****** These assignments are interchangeable.

EI-MS (70 eV): *m/z* (%) = 451 (100) [M^+], 408 (13) [M^+-$COCH_3$].

HR-EI-MS: *m/z* (%) = 451.1620 (M^+, calcd 451.1631 for $C_{25}H_{25}NO_7$).

5,8-Diacetoxy-7-(ethyl-phenyl-amino)-1,4-dihydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester



94b

Compound **94b** was synthesized according to *GWP4* starting from intermediate **93** (0.06 g, 0.17 mmol) and acetic anhydride (0.11 ml, 1.23 mmol). The crude product was purified by flash chromatography (eluent: toluene/EtOAc, 9:1) to provide the title compound in 11 % yield as pale yellow oil.

R_f = 0.32 (toluene/EtOAc = 9:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.12 (t, ^{3}J (H-13, H-12) = 8.9 Hz, 2H, H-13), 6.83 (m, ^{3}J (H-16, H-17, H-15) = 5.3 Hz, H-16), 6.80 (m, ^{3}J (H-17, H-18, H-15) = 5.3 Hz, 1H, H-17) 6.72 (t, 1H, H-14), 6.60 (d, ^{3}J (H-12, H-13)= 8.9 Hz, 2H, H-12), 3.91 (m, 1H, H-15), 3.81 (m, 1H, H-18), 3.60 (s, 3H, H-8), 3.59 (q, 2H, H-9), 2.29 (s, 3H, H-20*), 2.22 (m, 1H, H-19a), 2.20 (m, 1H, H-19b), 1.91 (s, 3H, H-23*), 1.20 (t, 3H, H-10).

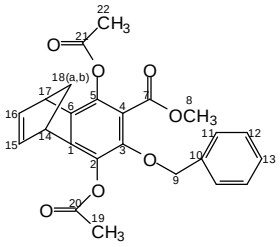
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 168.44 (C-22*), 168.11 (C-21*), 165.69 (C-7), 148.84 (C-1**), 147.65 (C-11), 143.93 (C-6**), 142.39 (C-16***), 142.23 (C-17***), 140.56 (C-5), 139.26 (C-2), 135.95 (C-3), 128.74 (C-13), 125.43 (C-4), 118.02 (C-12), 114.58 (C-14), 52.22 (C-8), 48.97 (C-15****), 48.27 (C-18****), 46.79 (C-9), 20.61 (C-23*****), 20.28 (C-20*****), 12.82 (C-10).

*, **, ***, ****, ***** These assignments are interchangeable.

ESI-MS: *m/z* (%) = 458 [M^++ Na], 436 [M^+ + H].

HR-ESI-MS: *m/z* (%) = 436.1770 (M^+ + H, calcd 436.1760 for $C_{25}H_{26}NO_6$).

5,8-Diacetoxy-7-benzyloxy-1,4-dihydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester



94c

The title compound **94c** was synthesized according to *GWP4* from intermediate **93** (0.10 g, 0.29 mmol) and acetic anhydride (0.11 ml, 1.23 mmol). The crude product was purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: DCM/EtOAc, 9:1) to provide the title compound in 96 % yield as yellow oil.

R_f = 0.90 (DCM/EtOAc = 9:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.30 (m, 5H, H-11, H-12, H-13), 6.81 (m, ^{3}J (H-15, H-16, H-14) = 5.1 Hz, 1H, H-15*), 6.76 (m, ^{3}J (H-16, H-17, H-15) = 5.1 Hz, 1H, H-16*), 4.96 (s, 2H, H-9), 3.88 (m, 2H, H-17**, H-14**), 3.76 (s, 3H, H-8), 2.28 (s, 3H, H-22***), 2.21 (m, 2H, H-18), 2.17 (s, 3H, H-19***).

*, **, *** These assignments are interchangeable.

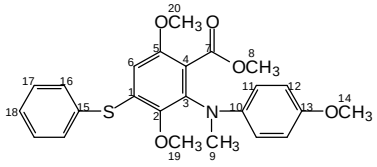
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 168.57 (C-21*), 168.35 (C-20*), 164.81 (C-7), 148.65 (C-6**), 147.47 (C-3), 142.67 (C-15***), 141.72 (C-16***), 140.58 (C-1**), 139.02 (C-5****), 137.17 (C-10), 136.49 (C-2****), 128.37 (C-12******), 127.94 (C-13), 127.57 (C-11*****), 118.38 (C-4), 76.95 (C-9), 67.78 (C-18), 52.24 (C-8), 48.43 (C-17******), 47.82 (C-14******), 20.54 (C-19*******), 20.41 (C-22*******).

*, **, ***, ****, *****, ******, ******* These assignments are interchangeable.

EI-MS (70 eV): *m/z* (%) = 422 (100) [M^+], 391 (17) [M^+ -OCH_3], 91 (84) [$CH_2C_6H_5$].

HR-EI-MS: *m/z* (%) = 422.1355 (M^+, calcd 422.1365 for $C_{24}H_{22}O_7$).

3,6-Dimethoxy-2-(N-methyl-4`-anisidino)-4-phenyl sulfanyl benzoic acid methyl ester synthesis



96a

The title compound **96a** was synthesized according to *GWP2* starting from 3,6-dihydroxy-2-(N-methyl-4`-anisidino)-4-phenyl sulfanyl benzoic acid methyl ester **95** (0.03 g, 0.07 mmol) and MeI (0.05 ml, 0.84 mmol). The crude product was purified by preparative layer chromatography (eluent: toluene/EtOAc, 9:1) followed by crystallization in methanol to provide the title compound in 90 % yield as white needles.

R_f = 0.40 (toluene/EtOAc = 9:1)

m.p. = 102-103 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.48 (m, ^{3}J (H-16, H-17) = 8.0 Hz, 2H, H-16), 7.41 (t, 1H, H-18), 7.39 (d, ^{3}J (H-17, H-16) = 8.0 Hz, 2H, H-17), 6.74 (d, ^{3}J (H-12, H-11) = 9.0 Hz, 2H, H-12), 6.64 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 6.30 (s, 1H, H-6), 3.73 (s, 3H, H-14), 3.61 (s, 3H, H-19), 3.53 (s, 3H, H-20), 3.47 (s, 3H, H-8), 3.17 (s, 3H, H-9).

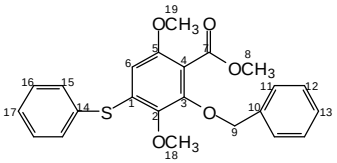
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 166.27 (C-7), 153.21 (C-5), 152.28 (C-13), 148.56 (C-2), 142.59 (C-10), 140.12 (C-3), 135.65 (C-1), 133.65 (C-16), 132.62 (C-15), 129.57 (C-17), 128.49 (C-18), 122.00 (C-4), 115.31 (C-11*), 114.34 (C-12*), 109.31 (C-6), 60.78 (C-20), 56.06 (C-19), 55.72 (C-14), 52.04 (C-8), 38.99 (C-9).

* These assignments are interchangeable.

EI-MS (70eV): *m/z* (%) = 439 (100) [M^+], 424 (38) [M^+ -CH_3], 378 (25) [M^+ -OCH_3, -$2CH_3$], 149 (97) [$C_8H_5O_3$].

HR-EI-MS: *m/z* (%) = 439.1444 (M^+, calcd 439.1453 for $C_{24}H_{25}NO_5S$).

2-Benzyloxy-3,6-dimetoxy-4-phenyl sulfanyl-benzoic acid methyl ester



96b

Alkylated compound **96b** was synthesized according to *GWP2* starting from hydroquinone **95** (0.03 g, 0.11 mmol) and MeI (0.08 ml, 1.21 mmol). The crude product was purified by preparative layer chromatography on Merck silica gel (60 F_{254}) plates (eluent: PEE/EtOAc, 8:2) followed by crystallization in MeOH to provide the title compound in 96 % yield as white needles.

R_f = 0.46 (PEE/EtOAc = 8:2)

m.p. = 93-94 °C

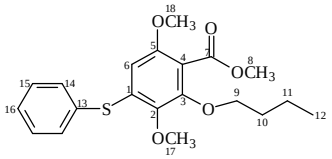
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.47-7.37 (m, 10H, H-11, H-12, H-13, H-15, H-16, H-17), 6.19 (s, 1H, H-6), 5.10 (d, 2H, H-9), 3.85 (s, 3H, H-18), 3.79 (s, 3H, H-8), 3.53 (s, 3H, H-19).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 107.14 (C-6), 165.97 (C-7), 152.79 (C-5), 149.75 (C-3), 144.23 (C-2), 137.02 (C-10), 135.0 (C-1), 133.50 (C-15), 130.31 (C-14), 129.50 (C-12, C-16), 128.40, 128.20 (C-13, C-17), 117.23 (C-4), 128.50 (C-11), 76.68 (C-9), 60.9 (C-19), 56.1 (C-18), 52.50 (C-8).

EI-MS (70 eV): *m/z* (%) = 410 (97) [M^+], 379 (8) [M^+ -OCH_3], 269 (95) [M^+ -SHC_6H_5, -OCH_3], 91 (100) [M^+ -$CH_2C_6H_5$].

HR-EI-MS: *m/z* (%) = 410.1188 (M^+, calcd 410.1149 for $C_{23}H_{22}O_5S$).

2-Butoxy-3,6-dimethoxy-4-phenyl sulfanyl-benzoic acid methyl ester



96c

Compound **96c** was synthesized according to *GWP2* starting from hydroquinone **95** (0.02 g, 0.06 mmol) and MeI (0.04 ml, 0.74 mmol) to provide the title compound in a 100 % yield as yellow oil.

R_f = 0.72 (hexane/EtOAc = 8:2)

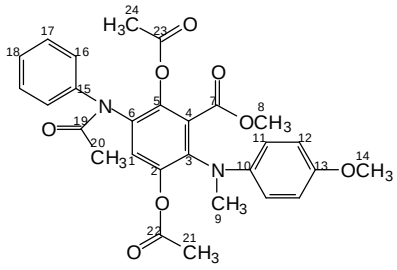
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.49-7.33 (m, 5H, H-14, H-15, H-16), 6.15 (s, 1H, H-6), 4.11 (t, 2H, H-9), 3.85 (s, 3H, H-8), 3.81 (s, 3H, H-17), 3.52 (s, 3H, H-18), 1.72-1.62 (m, 2H, H-10), 1.49-1.40 (m, 2H, H-11), 0.95 (t, 3H, H-12).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 166.15 (C-7), 152.71 (C-5), 150.18 (C-3), 144.27 (C-2), 134.70 (C-1), 133.40 (C-14), 132.88 (C-13), 129.50 (C-16), 128.33 (C-16), 117.28 (C-4), 106.89 (C-6), 74.17 (C-9), 60.69 (C-17), 56.07 (C-18), 52.41 (C-8), 32.29 (C-10), 19.13 (C-11), 13.86 (C-12).

EI-MS (70 eV): *m/z* (%) = 376 (100) [M^+], 345 (12) [M^+ -OCH_3], 288 (71) [M^+ -$O(CH_2)_3CH_3$], 273 (25) [M^+ -$O(CH_2)_3CH_3$, -CH_3].

HR-EI-MS: *m/z* (%) = 376.1331 (M^+, calcd 376.1344 for $C_{20}H_{24}O_5S$).

2,5-Diacetoxy-3-(acetyl-phenyl-amino)-6-(N-methyl-4`-anisidino)-benzoic acid methyl ester



100

A mixture of **93b** (0.02 g, 0.05 mmol), triethylamine (0.02 ml, 0.15 mmol), acetic anhydride (0.01 ml, 0.15 mmol) and (1.00 mg, 8.00 μmol) of DMAP is allowed to stand for 14 h at 24 °C. Afterwards the reaction mixture was evaporated in vacuum, and the residue was purified

by preparative layer chromatography (eluent: CH_2Cl_2/EtOAc, 9:1) to afford the title compound in 90 % as yellow solid.

R_f = 0.62 (DCM/EtOAc = 9:1)

m.p. = 74-75 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.35 (m, 5H, H-18, H-17, H-16), 6.74 (d, ^{3}J (H-12, H-11) = 9.0 Hz, 2H, H-12), 6.64 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 5.29 (s, 1H, H-1), 3.72 (s, 3H, H-14), 3.51 (s, 3H, H-8), 3.14 (s, 3H, H-9), 2.16 (d, 2H, H-21, H-24), 2.00 (s, 3H, H-20).

The ^{13}C-NMR could not be analysing because:

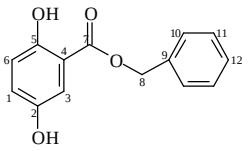
- Broad lines at 304 K are due to hindered rotation about the Ph_2NCOCH_3 bonds.
- Heating the solution to 353 K in $(CD_3)_2SO$ causes faster rotation, more averaging of environments and here sharper lines.

EI-MS (70 eV): *m/z* (%) = 520 (100) [M^+], 478 (72) [M^+ -$COCH_3$], 462 (18) [M^+ -$COCH_3$, -CH_3].

HR-EI-MS: *m/z* (%) = 520.1856 (M^+, calcd 520.1846 for $C_{28}H_{28}N_2O_8$).

8.1.5 Compounds from Chapter 4

2,5-Dihydroxybenzoate



106

To a solution of 2,5-dihydroxybenzoic acid **(102)** (2.00 g, 12.96 mmol) in 80 ml acetone was slowly added benzyl chloride (1.73 g, 14.28 mmol) in 8 ml acetone and K_2CO_3 (1.97 g, 14.28 mmol). After 12 h of stirring at 70 °C, the suspension was cooled to 20 °C and filtered. The solvent was evaporated in vacuo at 40 °C. The residue was dissolved in EtOAc and purified by chromatography (eluent: toluene/EtOAc, 9:1) to provide the title compound in 50 % yield as colourless crystals.

R_f = 0.23 (toluene/EtOAc = 9:1)

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 10.34 (s, 1H, HO-2), 7.36-7.44 (5H, m, H-10, H-11, H-12), 7.32 (d, ^{4}J (H-1, H-3) = 2.8 Hz, 1H, H-3), 6.99 (dd, ^{3}J (H-1, H-6) = 8.9 Hz, ^{4}J (H-1, H-3) = 2.8 Hz, 1H, H-1), 6.88 (d, ^{3}J (H-1, H-6) = 8.9 Hz, 1H, H-6), 5.36 (s, 2H, H-8).

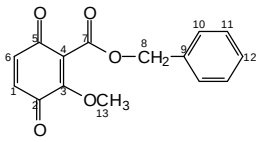
^{13}C-NMR (151 MHz, $CDCl_3$): δ = 169.51 (C-7), 156.1 (C-5), 147.68 (C-2), 135.22 (C-9), 128.76 (C-10), 128.64 (C-12), 128.35 (C-11), 124.20 (C-1), 118.61 (C-6), 114.80 (C-3), 112.21 (C-4), 67.13 (C-8).

EI-MS *m/z* (%) = 244 (100) [M^+], 153 (8) [M^+ -$C_6H_5CH_2$], 136 (39) [M^+ -$C_7H_4O_3$], 92 (68) [C_7H_8].

HR-EI-MS: *m/z* (%) = 244.0733 (M^+, calcd 244.0735 for $C_{14}H_{12}O_4$).

The spectroscopic data is in agreement with the assigned structure and those reported by Cavallito and Buck.[66]

2-Benzyloxycarbonyl-1,4-benzoquinone



108

To a solution of 2,5-dihydroxybenzoate (0.02 g, 0.08 mmol) in 0.50 ml methanol was added in one portion DDQ (21.48 g, 0.08 mmol). After being stirred at room temperature overnight, the reaction solution was evaporated under reduced pressure and the residue was purified by preparative layer chromatography (eluent: toluene/EtOAc, 9:1) to provide the title compound in 10 % yield.

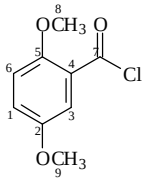
R_f = 0.88 (toluene/EtOAc = 9:1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.40-7.31 (m, 5H, H-10, H-11, H-12), 6.70 (d, ^{3}J (H-6, H-1) = 10.2 Hz, ^{3}J (H-6, H-1) = 10.2 Hz, 2H, H-1, H-6), 5.34 (s, 2H, H-8) 3.92 (s, 3H, H-13).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 184.51 (C-2), 182.01 (C-5), 163.47 (C-7), 153.61 (C-3), 136.40 (C-6), 134.68 (C-1), 128.15 (C-9), 127.89-128.72 (C-10, C-11, C-12), 118.84 (C-4), 68.15 (C-8), 59.4 (C-13).

ESI-MS: *m/z* (%) = 295 (100) [M^+ + Na], 273 (10) [M^+ + H].

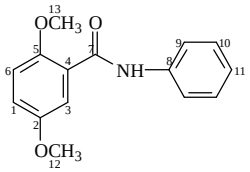
2,5-Dimethoxy-N-phenyl-benzamide[68]



115

Oxalyl chloride (0.55 ml, 6.26 mmol) was added dropwise to a stirred suspension of 2,5-dimethoxybenzoic acid (0.87 g, 4.84 mmol) in 4 ml toluene and drops of DMF. As the reaction progressed, the acid gradually dissolved gave a clear-yellow solution. After 2.5 h, the volatiles were removed on the rotary evaporator to give the title product as pale-yellow oil, which was used immediately in the next step without further purification.

2,5-Dimethoxy-N-phenyl-benzamide



120

To a solution of **115** (0.86 g, 4.30 mmol) in 20 ml acetonitrile was added aniline (0.33 ml, 4.30 mmol) and K_2CO_3 (0.77 g, 5.60 mmol). After being stirred at room temperature overnight, the reaction solvent was evaporated and the residue redissolved in ether and washed with 1N NaOH solution. The organic phase was evaporated and the residue recrystallized from ether to give the title product in 53 % yield as white crystals.

R_f = 0.11 (DCM)

m.p. = 96-97 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.95 (s, 1H, NH), 7.88 (d, ^{4}J(H-1, H-3) = 3.3 Hz, 1H, H-3), 7.71 (d, ^{3}J(H-9, H-10) = 8.5 Hz, 2H, H-9), 7.37 (t, ^{3}J(H-10, H-9) = 8.5 Hz, 2H, H-10), 7.14 (t, 1H, H-11), 7.09 (dd, ^{3}J(H-1, H-6) = 8.8 Hz, 1H, H-1), 6.98 (d, ^{3}J(H-6, H-1) = 8.8 Hz, 1H, H-6), 4.00 (s, 3H, H-13), 3.85 (t, 3H, H-12).

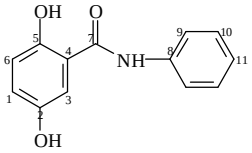
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 162.99 (C-7), 154.26 (C-2), 152.56 (C-5), 138.44 (C-8), 129.03 (C-10), 124.21 (C-11), 122.47 (C-4), 120.44 (C-9), 119.98 (C-1), 115.69 (C-3), 113.42 (C-6), 56.95 (C-12), 55.90 (C-13).

ESI-MS: *m/z* (%) = 280 [M^+ + Na], 258 [M^+ + H].

HR-ESI-MS: *m/z* (%) = 258.1140 (M^+ + H, calcd 258.1130 for $C_{15}H_{16}NO_3$).

The spectroscopic data is in good agreement with the assigned structure and those reported by Bäckwall *et al*.[59]

2,5-Dihydroxy-N-phenyl-benzamide



121

To a cooled (-60 °C) solution of 2,5-Dimethoxy-N-phenyl-benzamide (0.17 g, 0.66 mmol) **(120)** in 1 ml dry DCM under N_2 was added BBr_3 (0.25 ml, 0.26 mmol). The reaction mixture was stirred for 30 minutes at –60 °C, followed by stirring 4 h at room temperature. Then the excess of BBr_3 was neutralized with MeOH, the solvent was evaporated, the residue redissolved in EtOAc and extracted with saturated solution of $NaHCO_3$. Then the organic phase was evaporated and the residue purified by chromatography (eluent DCM / MeOH, 9:1) in 96 % yield as white solid.

R_f = 0.49 (DCM/MeOH = 9:1)

m.p. = 162-163 °C

^{1}H-NMR (400 MHz, CD_3OD): δ = 11.34 (OH), 7.83 (NH), 7.71 (d, ^{3}J(H-9, H-10) = 8.5 Hz, 2H, H-9), 7.42 (d, ^{4}J(H-3, H-1) = 4.0 Hz, 1H, H-3), 7.38 (t, ^{3}J(H-10, H-9) = 8.5 Hz, 2H, H-10), 7.20 (t, 1H, H-11), 6.98 (dd, ^{4}J(H-1, H-3) = 4.0 Hz, ^{3}J (H-1, H-6) = 10.4 Hz, 1H, H-1), 6.87 (d, ^{3}J(H-6, H-1) = 10.4 Hz, 1H, H-6).

^{13}C-NMR (100 MHz, CD_3OD): δ = 168.02 (C-7), 152.64 (C-5), 151.30 (C-2), 139.38 (C-8), 129.89 (C-10), 125.64 (C-11), 122.43 (C-1), 122.31 (C-9), 118.99 (C-4), 118.92 (C-6), 115.76 (C-3).

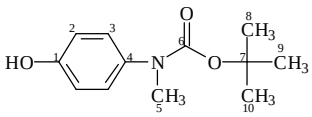
ESI-MS: *m/z* (%) = 252 [M^+ + Na], 230 [M^+ + H].

HR-ESI-MS: *m/z* (%) = 230.0816 (M^+ + H, calcd 230.0817 for $C_{13}H_{12}NO_3$).

The spectroscopic data is in agreement with the assigned structure and those reported by Brimble *et al*.[76]

8.1.6 Compounds from Chapter 5

N-(*tert*-Butoxycarbonyl)-N-methylaminophenol



129

To a solution of N-methylaminophenol (1.00 g, 8.13 mmol) in 25 ml dioxane/water (2:1) was added di-tert-butyldicarbonate (1.77 g, 8.13 mmol) in two portions followed by stirring at room temperature 4-5 h. Dioxane was then removed in vacuum, the residue was cooled to 0 °C, and 20 ml EtOAc was added. The solution was acidified to pH 2-3 with $KHSO_4$ and extracted three times with EtOAc. The solvent was evaporated under reduced pressure to give the title compound in 100 % yield as brown solid.

m.p. = 132-133 °C

^{1}H-NMR (300 MHz, $(CD_3)_2SO$): δ = 9.32 (s, 1H, HO-1), 7.17 (d, ^{3}J (H-3, H-2) = 8.9 Hz, 2H, H-3), 6.72 (d, ^{3}J (H-2, H-3) = 8.9 Hz, 2H, H-2), 3.14 (s, 3H, H-5), 1.42 (s, 9H, H-8, H-9, H-10).

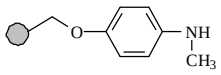
^{13}C-NMR (75 MHz, (CD_3)2SO): δ = 154.94 (C-6), 154.08 (C-1), 134.97 (C-4), 126.78 (C-3), 114.97 (C-2), 78.85 (C-7), 37.38 (C-5), 27.92 (C-8, C-9, C-10).

ESI-MS: *m/z* (%) = 469 [M^+ + Na], 246 [M^+ + Na], 224 [M^+ + H].

HR-EI-MS: *m/z* (%) = 223.1195 (M^+, calcd 223.1208 for $C_{12}H_{17}NO_3$).

The spectroscopic data is in agreement with the assigned structure and those reported by Fahrey *et al*.[73]

Synthesis of (4-benzyloxypolystyrene-phenyl)-methyl amine



130

To a solution of N-(tert-Butoxycarbonyl)-N-methylaminophenol **(129)** (10 equiv., 1.25 mmol, 0.28 g) in 1 ml dry DMF was added carefully NaH (10 equiv., 1.25 mmol, 0.03 g) washed free of petroleum oil. This slurry was added to the Merrifield resin (0.10 g, 1.25 mmol/g) together with KI (1 equiv., 0.125 mmol, 0.02 g). The reaction mixture was stirred gently overnight at 60 °C. At the end of this time, the solution was filtered off and the resin washed with DMF, DCM, MeOH, followed by Boc deprotection with 50 % TFA/DCM for 15-20 minutes and washing with DMF, DCM, triethylamine in DCM (1:1), MeOH and DCM to afford resin **(130)** in 93 % yield.

Loading was determined from the chlorine left unreacted on the resin: Boc protected amino resin (0.10 g, 0.12 mmol) was heated with pyridine (2 ml) in boiling water for 60 min. The solution with the resin was then transferred to an Erlenmeyer flask with 50 ml of 20 % acetic acid. A Volhard titration for chloride was carried out by addition of ferric amoniumsulfate indicator (3 drops), concentrated nitric acid (5 ml), 0.1 N $AgNO_3$ (5 ml), and toluene (3 ml), followed by back-titration with 0.1 N KSCN.[74]

$$\% Cl^- = 3.545\ (V_1N_1\text{-}V_2N_2)/m$$

V_1 = the added amount of silver nitrate solution (ml)

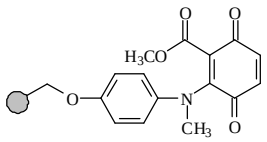
N_1 = the normality of silver nitrate solution

V_2 = the added amount of ammonium thiocyanate solution during the titration (ml)

N_2 = the normality of the ammonium thiocyanate solution

m = the weight of the sample (g)

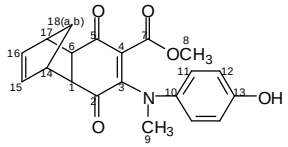
2-[(4-Benzyloxypolystyrene-phenyl)-methyl-amino]-3,6-dioxo-cyclohexa-1,4-diene-carboxylic acid methyl ester



132

To swollen resin **130** (0.10 g, 0.125 mmol) was added a solution of 1,4-benzoquinone **39** (6 equiv., 0.75 mmol, 0.12 g) followed by stirring overnight at room temperature. Afterwards the solution was filtered off and the dark violet resin was washed with DCM. Then DDQ (2 equiv., 0.25 mmol, 0.06 g) in 1 ml DCM was added to swollen resin. The mixture was stirred overnight at room temperature. At the end of this time the solution was filtered and the resin was washed with DCM to provide violet resin **132**.

7-[(4-Hydroxy-phenyl)-methyl-amino]-5,8-dioxo-1,4,4a,5,8,8a-hexahydro-1,4-methano-naphtalene-6-carboxylic acid methyl ester



135

To swollen resin **132** (0.10 g, 0.125 mmol) was added a solution of freshly cracked cyclopentadiene (40 equiv., 5 mmol, 0.30 g) in 1 ml toluene. The reaction mixture was stirred overnight at room temperature. At the end of this time the solution was filtered off and the orange resin was washed with DCM, MeOH and ether followed by drying to provide the resin **135**. Cleavage according to *GWP8* afforded the title compound in a 25 % yield as orange solid after purification by preparative layer chromatography (eluent: DCM/EtOAc, 9:1).

R_f = 0.28 (DCM/Ether = 7:3)

m.p. = 161-162 °C

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.94 (d, ^{3}J (H-11, H-12) = 9.0 Hz, 2H, H-11), 6.72 (d, ^{3}J (H-12, H-11) = 9.1 Hz, 2H, H-12), 6.22 (dd, ^{3}J (H-15, H-16, H-14) = 5.7 Hz, 1H, H-15), 6.12 (dd, ^{3}J (H-16, H-15, H-17) = 5.7 Hz, 1H, H-16), 5.60 (s, 1H, OH), 3.50 (m, 1H, H-14), 3.35 (m, 1H, H-17), 3.41 (s, 3H, H-8), 3.36 (dd, ^{3}J (H-6, H-17) = 9.2 Hz, ^{3}J (H-6, H-1) = 3.8 Hz, 1H, H-6), 3.28 (s, 3H, H-9), 3.25 (dd, ^{3}J (H-1, H-14) = 9.2 Hz, ^{3}J (H-1, H-6) = 3.8 Hz, 1H, H-1), 1.55 (t, 1H, H-18a), 1.40 (t, 1H, H-18b).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 196.98 (C-2*), 193.30 (C-5*), 166.69 (C-7), 155.41 (C-3), 154.78 (C-13), 138.34 (C-10), 136.76 (C-15**), 135.05 (C-16**), 127.12 (C-11), 118.41 (C-

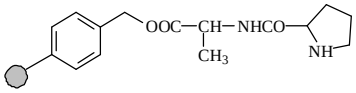
4), 116.18 (C-12), 52.00 (C-8), 50.27 (C-6), 49.96 (C-1), 48.80 (C-18), 47.75 (C-14***), 47.35 (C-17***), 44.13 (C-9).

*, **, *** These assignments are interchangeable.

ESI-MS (70 eV): *m/z* (%) = 355 (25) [M^+ + 2H], 354 (100) [M^+ + H], 353 (4) [M^+].

HR-ESI-MS: *m/z* (%) = 354.1340 (M^+ + H, calcd 353.1341 for $C_{20}H_{20}NO_5$).

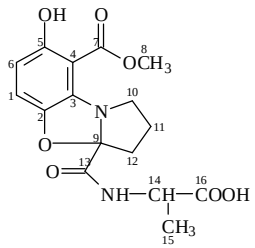
2-[(Pyrrolidine-2-carbonyl)-amino]-propionic acid benzylpolystyrene ester[76]



141

To the swollen N-Ala functionalized Merrifield resin synthesized according to *GWP9* (0.10 g, 0.125 mmol, 1 equiv.) was added a solution of N-Fmoc-Pro-OH **(138)** (0.62 mmol, 0.21 g), TBTU (0.62 mmol, 0.20 g) and DIPEA (1.25 mmol, 0.217 ml) in 1ml DMF. After stirring for 3 h at room temperature, the solution was filtered off, the resin washed with DMF, DCM, MeOH and dried under high vacuum overnight to provide resin **139** which was then Fmoc deprotected with 20 % piperidine in DMF for 15-20 minutes followed by washing with DCM and MeOH to provide the title resin 1.14 mmol/g (the loading was determined according to *GWP10*).

3a-(1-Carboxy-ethylcarbamoyl)-7-hydroxy-1,2,3,3a-tetrahydro-benzo [*d*] pyrrolo [2,1-*b*] oxazole-8-carboxylic acid methyl ester



143

A solution of 2-methoxycarbonyl-1,4-benzoquinone **39** (6 equiv., 0.75 mmol, 0.12 g) in 1 ml DCM was added to the swollen resin **141** (0.10 g, 0.125 mmol). The mixture was stirred overnight at room temperature. Afterwards the solution was filtered off and the resin was washed with DMF, DCM, MeOH followed by drying overnight under high vacuum to afford the purple resin **142**. Cleavage according to *GWP8* afforded the title compound in a 50 % yield as yellow-brown solid.

m.p. = 74-75 °C

R_f = 0.38 (DCM/MeOH = 8:2)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.60, 10.55 (s, 1H, HO-5), 7.59, 7.50 (d, 1H, NH), 6.89, 6.87 (d, ^{3}J (H-1, H-6) = 8.7 Hz, 1H, H-1), 6.51, 6.49 (d, ^{3}J (H-6, H-1) = 8.7 Hz, 1H, H-6), 5.57 (s, 1H, HO-16), 4.58-4.46 (m, 1H, H-14), 3.97 (s, 3H, H-8), 3.70, 3.21 (m, 1H, H-10), 2.44, 2.35 (m, 2H, H-12), 2.06, 1.94 (m, 2H, H-11), 1.44, 1.41 (d, 3H, H-15).

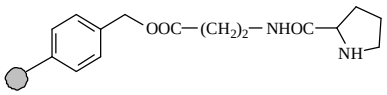
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 175.93, 175.85 (C-16), 170.61, 170.08 (C-13), 169.47, 169.42 (C-7), 156.71, 156.61 (C-5), 144.35, 144.26 (C-2), 140.32, 140.13 (C-3), 115.85,

115.03 (C-1), 110.78, 110.53 (C-6), 108.52, 108.42 (C-9), 102.75 (C-4), 59.54, 58.32 (C-10), 52.98, 52.53 (C-8), 48.15, 48.11 (C-14), 36.00, 35.85 (C-12), 24.50, 24.38 (C-11), 17.97, 17.93 (C-15).

ESI-MS: *m/z* (%) = 723 [$2M^+$ + Na], 373 [M^+ + Na], 351 [M^+ + H].

HR-EI-MS: *m/z* (%) = 350.1092 (M^+, calcd 350.1114 for $C_{16}H_{18}N_2O_7$).

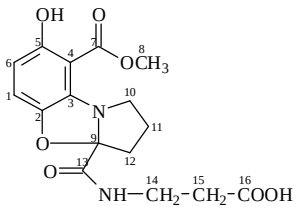
3-[(Pyrrolidine-2-carbonyl)-amino]-propionic acid benzylpolystyrene ester [76]



141

To the swollen N-β-Ala functionalised Merrifield resin synthesized according to *GWP9* (0.10 g, 0.125 mmol, 1 equiv.) was added a mixture of N-Fmoc-L-Pro and N-Fmoc-D-Pro (1:1), (0.62 mmol, 0.21 g), TBTU (0.62 mmol, 0.20 g) and DIPEA (1.25 mmol, 0.217 ml) in 1 ml DMF. After stirring for 3 h at room temperature, the solution was filtered off, the resin washed with DMF, DCM, MeOH and dried under high vacuum overnight to provide resin **139** which was then Fmoc deprotected with 20 % piperidine in DMF for 15-20 minutes followed by washing with DCM and MeOH to provide the title resin 1.12 mmol/g (the loading was determined according to *GWP10*).

3a-(2-Carboxy-ethylcarbamoyl)-7-hydroxy-1,2,3,3a-tetrahydro-benzo [*d*] pyrrolo [2,1-*b*] oxazole-8-carboxylic acid methyl ester



143

A solution of 2-methoxycarbonyl-1,4-benzoquinone **39** (6 equiv., 0.75 mmol, 0.12 g) in 1ml DCM was added to the swollen resin **141** (0.10 g, 0.125 mmol). The mixture was stirred overnight at room temperature. Afterwards the solution was filtered off and the resin was washed with DMF, DCM, MeOH followed by drying overnight under high vacuum to afford the purple resin **143**. Cleavage according to *GWP8* afforded the title compound in 40-50% yield as yellow-brown solid.

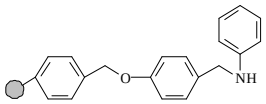
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.58 (s, 1H, OH), 7.58 (t, 1H, NH), 6.86 (d, ^{3}J (H-1, H-6) = 8.8 Hz, 1H, H-1), 6.46 (d, ^{3}J (H-6, H-1) = 8.8 Hz, 1H, H-6), 3.96 (s, 3H, H-8), 3.65 (ddd, ^{2}J (H-10A, H-10B) = 11.0 Hz, ^{3}J (H-10A, H-11) = 8.0, 6.0 Hz, 1H, H-10A), 3.50 (dt, ^{3}J (H-14, NH) = 6), ^{3}J (H-14, H-15) = 6.1 Hz, 2H, H-14), 3.19 (ddd, ^{2}J (H-10A, H-10B) = 11 Hz, ^{3}J (H-10B, H-11) = 7.0, 4.5 Hz, 1H, H-10B), 2.55 (t, ^{3}J (H-15, H-14) = 6.0 Hz, 2H, H-15), 2.47 (ddd, ^{2}J (H-12A, H-12B) = 13.5 Hz, ^{3}J (H-12A, H-11) = 9.0, 7.0 Hz, 1H, H-12A), 2.34 (ddd, ^{2}J (H-12B, H-12A) = 13.5 Hz, ^{3}J (H-12B, H-11) = 7.5, 5.5 Hz), 1H, H-12B), 1.95 (m, 2H, H-11).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 176.69 (C-16), 170.75 (C-13), 169.48 (C-7), 156.57 (C-5), 144.42 (C-2), 140.32 (C-3), 114.86 (C-1), 110.54 (C-6), 108.69 (C-9), 102.74 (C-4), 58.23 (C-10), 52.51 (C-8), 35.72 (C-12), 34.60 (C-14), 33.54 (C-15), 24.47 (C-11).

ESI-MS: *m/z* (%) = 373 [M^+ + Na], 351 [M^+ + H].

HR-ESI-MS: *m/z* (%) = 351.0930 (M^+ + H calcd 351.1192 for $C_{16}H_{19}N_2O_7$).

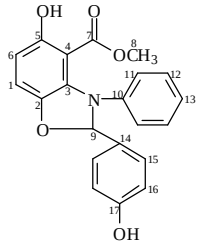
(4-Benzylpolystyrene-benzyl)-phenyl amine[80]



149

To a suspension of bromo-Wang resin **(148)** (0.50 g, 1.60 mmol/g) in 11 ml dried DCM was added aniline (0.73 ml, 8.00 mmol). The suspension was gently stirred at room temperature overnight. Then the mixture was transferred to a syringe reactor and washed successively with THF-H_2O (3:2, 3x10 ml), H_2O (3x10 ml), THF (3x10 ml), DCM (3x10 ml), to give the title resin **149**.

5-Hydroxy-2-(4-hydroxy-phenyl)-3-phenyl-2,3-dihydro-benzooxazole-4-carboxylic acid methyl ester



151

To the swollen amino resin **149** (0.50 g, 1.60 mmol/g) was added a solution of 2-methoxycarbonyl-1,4-benzoquinone (0.72 mmol, 4.36 mmol) in 3 ml dried toluene followed by stirring at room temperature overnight. Then, the resin was filtered off and washed with DCM followed by drying under reduced pressure. The resin was then treated with TMSOTf (10 equiv., 1.45 ml, 8.00 mmol) in 27 ml dry DCM at room temperature for 3 h. At the end of this time, a saturated aqueous solution of $NaHCO_3$ was added. The aqueous layer was extracted with DCM. The combined organic layer was washed with aqueous NaCl and dried over $MgSO_4$. After removal of the solvent, the residue was purified by preparative layer chromatography (PLC) on Merck silica gel (60 F_{254}) plates (eluent: toluene/EtOAc, 9:1) to provide the title compound in 1.3 % yield as yellow oil.

R_f = 0.40 (toluene/EtOAc = 9:1)

^{1}H-NMR (300 MHz, CD_3OD): δ = 10.13 (s, 1H, OH-5), 7.45 (d, ^{3}J (H-15, H-16) = 8.7 Hz, 2H, H-15), 7.22 (t, ^{3}J (H-12, H-11) = 7.9 Hz, 2H, H-12), 7.07 (t, 1H, H-13), 6.89-6.5 (m, 5H, H-11, H-16, H-1), 6.51 (s, 1H, H-9), 6.45 (d, ^{3}J (H-6, H-1) = 8.7 Hz, 1H, H-6), 3.27 (s, 3H, H-8).

^{13}C-NMR (75 MHz, CD_3OD): δ = 169.16 (C-7), 156.64 (C-17), 155.30 (C-5), 146.97 (C-10), 144.59 (C-2), 135.44 (C-3), 131.90 (C-14), 129.14 (C-12), 128.60 (C-15), 124.46 (C-13), 120.56 (C-11), 115.62 (C-16), 114.65 (C-1), 108.79 (C-6), 102.10 (C-9), 51.25 (C-8).

ESI-MS: *m/z* (%) = 386 [M^+ + Na], 364 [M^+ + H].

HR-ESI-MS: *m/z* (%) = 364.1170 (M^+ + H, calcd 364.1184 for $C_{21}H_{18}NO_5$).

8.2 Biological Materials and Methods

8.2.1 Materials

Laboratory equipment and material

Pipettes: Glass pipettes sterilized at 180 °C, 4 h.

Cell culture flasks: Tissue culture flask, 50 ml, polystyrene (Becton Dickinson, U.S.A)

96-well plate: Microtest 96 (Becton Dickinson, U.S.A)

Microscope: Axiophot 35M (Zeiss, Jena, Germany)

The UV-absorption measurements for Cell Proliferation assay were run on a Titertek Multiscan MCC/340

Sarstedt: Petri dishes

Nalge Nunc: Four well plates

Chemicals

All chemicals were purchased from the following suppliers and used without further purification:

Cambrex: Dubelco`s MEM; Fetal bovine serum (FBS)

Marcor: caseine peptone

Difco: proteose peptone; yeast extract; malt extract

Merck: meat extract; NaOH

Roth: HEPES; HCl

Sigma: MTT solution

J.T.Baker: isopropanol; acetone; methanol

Sigma: DAPI

GIBCO: trypsin, PBS

Cell lines

L-929 mouse fibroblasts (ACC2) and PtK2 (ATCC CCL-56) cell line were provided by Deutsche Sammlung von Mikroorganisme und Zellkulturen and American Type Culture Collection.

Antibodies

Affinity BioReagents: monoclonal antibody against GRP-94; polyclonal antibody against ?-tubulin

Babco: goat anti-rat immunoglobulin G antibody conjugated with Alexa Fluor 488; goat anti-rabbit IgG antibody conjugated with Alexa Fluor 594.

Media and Reagents

Growth Medium (EBS)

5 g/l caseine peptone

5 g/l proteose peptone

1 g/l meat extract

1 g/l yeast extract

10 g/l HEPES, adjust pH 7.0 with 5M NaOH

Growth Medium 90

30 g/l malt extract

3 g/l caseine peptone, adjust pH 5.6 with 1M HCl

Staining Reagent

Azur B

8.2.2 Methods

Note: all procedures from this point on were performed using sterile technique.

Agar diffusion assay

Bacteria to be tested (*Staphylococcus aureus, Mycobacterium phlei, Micrococcus luteus, E. coli* tolC*, Pseudomonas aeruginosa, Klebsiella pneumoniae*) were grown in standard EBS medium (5 g/l caseine peptone, 5 g/l proteose peptone, 1 g/l meat extract, 1 g/l yeast extract, 10 g/l HEPES, pH 7.0) overnight. An aliquot of the cultures were seeded into molten EBS agar (1.5 %) medium to give an O.D. of 0.01. 15-ml aliquots of the still liquid medium were poured into Petri dishes. When the medium had solidified a paper disc of 6 mm diameter soaked with 20 µl of the compound solution to be tested were placed on the agar and the Petri dishes were incubated at 30 °C.

Assays with fungi were done in same way except that they were grown in medium 90 (30g/l malt extract, 3 g/l caseine peptone, pH 5.6). Yeasts (*Candida albicans, Hansenula anomala, Saccharomyces cerevisiae*) were seeded into the agar medium to give an O.D. of 0.1. With hyphal fungi (*Aspergillus fumigatus, Botrytis cinerea, Pythium debaryanum*), conidia collected from grown agar cultures were seeded into the assay agar plates according to experience.

The diameter of inhibition zones were measured after 1-2 days and given as a parameter for the antibiotic activity of a compound.

Minimal inhibitory concentration (MIC) test

120 µl of *Saccharomyces cerevisiae* culture, which was diluted to an O.D. of 0.15 was added to 60 µL of serial dilutions of the compounds in a 96-well microplate and incubated at 30 °C

overnight. The O.D. of the wells of the microplate was measured at 595 nm by a plate reader. The concentration range tested was normally 370 µg/ml down to 6 ng/ml.

Cytotoxicity assay

L-929 mouse fibroblasts cells were grown for 6-8 days at 37 °C, 10 % CO_2 in Dulbecco`s modified Eagle`s medium/high glucose (DMEM) supplemented with 10% (v/v) fetal calf serum and glutamine. The medium was removed and cells washed with EBBS twice. Then the cells were detached from there substrate by incubation with trypsine for 5-10 min and resuspended in new medium to a final density of 50.000 cells/ml. The cells (120 µl) were added to a serial dilution of the test compounds in 96-well plates (6000 cells/well). After 5 days of incubation, growth was observed under the microscope and determined by an MTT assay. The normal concentration of the compounds tested ranged from 370 µg/ml down to 6 ng/ml.

MTT assay

A solution of MTT (5mg/ml) was added to 96-well plates (20 µl/well) and incubated at 37 °C, 10 % CO_2 for 2 h. Then the plates were centrifuged, washed with PBS (100 µl/well), and the precipitated formazan crystals dissolved with a solution of 0.04N HCl in isopropanol (100 µl/well). After shaking the plates for 15 min the absorbance values measured at 595 nm in a microplate reader were taken as parameters of growth and vitality of the cells.

Cell staining and fluorescence labelling

PtK_2 cells were grown on glass cover slips (13 mm diameter) in four well plates to semi-confluent density and incubated with the compounds to be tested. At the end of incubation

time the supernatant medium was removed and cells were fixed with cold (-20 °C) acetone-methanol (1:1) for 10 min. For morphological studies cells were stained by azure B.

For labelling the endoplasmatic reticulum (ER) cells were incubated with a primary monoclonal antibody against GRP-94 (1:1000; Affinity BioReagents, Golden, U.S.A), and a secondary goat anti-rat immunoglobulin G antibody conjugated with Alexa Fluor 488 (1 µg/ml; Molecular Probes, Leiden, The Netherlands) at 37 °C, each for 1 hour.

For labelling the centrosomes, we used polyclonal antibodies against ?-tubulin (Babco) and a secondary goat anti-rabit IgG antibody conjugated with Alexa Fluor 594 (Molecular Probes).

Cells were rinsed with phosphate-buffered saline (GIBCOBRL, Eggenstein, Germany) between two incubations.

The nuclei were stained with DAPI (4',6-Diamino-2-phenylindole; 1µg/ml).

After staining the coverslips were mounted using Prolong Antifade (Molecular Probes), and examined with a Zeiss Axiophot fluorescence microscope (Carl Zeiss, Jena, Germany) using appropriate filter sets.

Abbreviations

11-b-HSD-1	11-β-Hydroxysteroid dihydrogenase type 1
11-b-HSD-2	11-β-Hydroxysteroid dihydrogenase type 2
Ac_2O	Acetic anhydride
Boc	*Tert*-butyl carboxycarbonyl
Cdc25A	Cell cycle-dependant kinase A
CDK	Cyclin-dependant kinase
DCM	Dichlormethane
DDQ	2,3-Dichloro-5,6-dicyano-p-benzoquinone
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
EtOAc	Ethyl acetate
EI	Electron impact
ESI	Electron spray ionization
Fmoc	9-Fluorenylmethyloxycarbonyl
IC_{50}	50% inhibitory concentration
MAP	Mitogen activated protein
MEK	Mitogen activating potein kinase
MeOH	Methanol
MIC	Minimal inhibition concentration
MS	Mass spectrum
NMR	Nuclear magnetic resonance
PEE	Petroleum ether
PLC	Preparative layer chromatography
R_f	Retention factor

RT	Room temperature
TBTU	O-(Benzotriazol-1-yl)-N,N,N`,N`-tetramethyluronium tetrafluoroborate
TEA	Trietyl amine
TFA	Trifluoro acetic acid
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyl triflate
VHR	Vaccinia virus H1-related phosphatase

9 References

Appendix

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Zusammenfassung
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„Kombinatorische Synthese von Bibliotheken niedermolekularer Verbindungen um das p-Benzochinon-Grundgerüst“

Die aus Chinon-Grundgerüst abgeleitete Naturstoffe sind für ihre zahlreichen biologischen Äktivitäten bekannt. Aus dem Grund stellt das 2-Methoxycarbonyl-1,4-benzochinon ein geeignetes Templat für die Synthese von Bibliotheken niedermolekularer Verbindungen für die chemisch genetischen Studien dar.

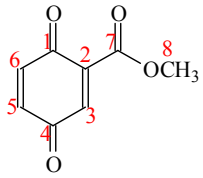


Abb. 1: Struktur von 2-Methoxycarbonyl-1,4-benzochinon

Das Ziel der vorliegenden Arbeit war die Entwicklung eines effizienten Synthesewegs für die Darstellung von p-Benzochinon-Bibliotheken unter Einbezug des Micheal-Addition-Konzeptes (siehe Abb. 2). Im ersten Schritt erfolgt die regioselektive Addition von verschiedenen Nuckleophilen am Benzequinonering unter Bildung von Hydrochinon als Zwischenstufe, welches in einem zweiten Schritt zu dem entsprechenden Chinon oxidiert wird. Unter diesen Reaktionsbedingungen (Oxidation und Reduktion) wurden zahlreiche Subtituenten an modifiziebaren Postionen am 1,4-Benzochinonering eingeführt. In einem weiteren Schritt wurden die gebildeten Hydochinone bzw. Chinone durch Alkylierung- bzw. Aceteylierungsreaktionen modifiziert.

Um die Machbarkeit dieser Strategie und das Potenzial des 2-Methoxycarbony-1,4-benzochinons als Grundgerüst zu demonstrieren, wurden die ersten Benzochinonederivative in der Lösung synthetisiert (*siehe Abb. 2*).

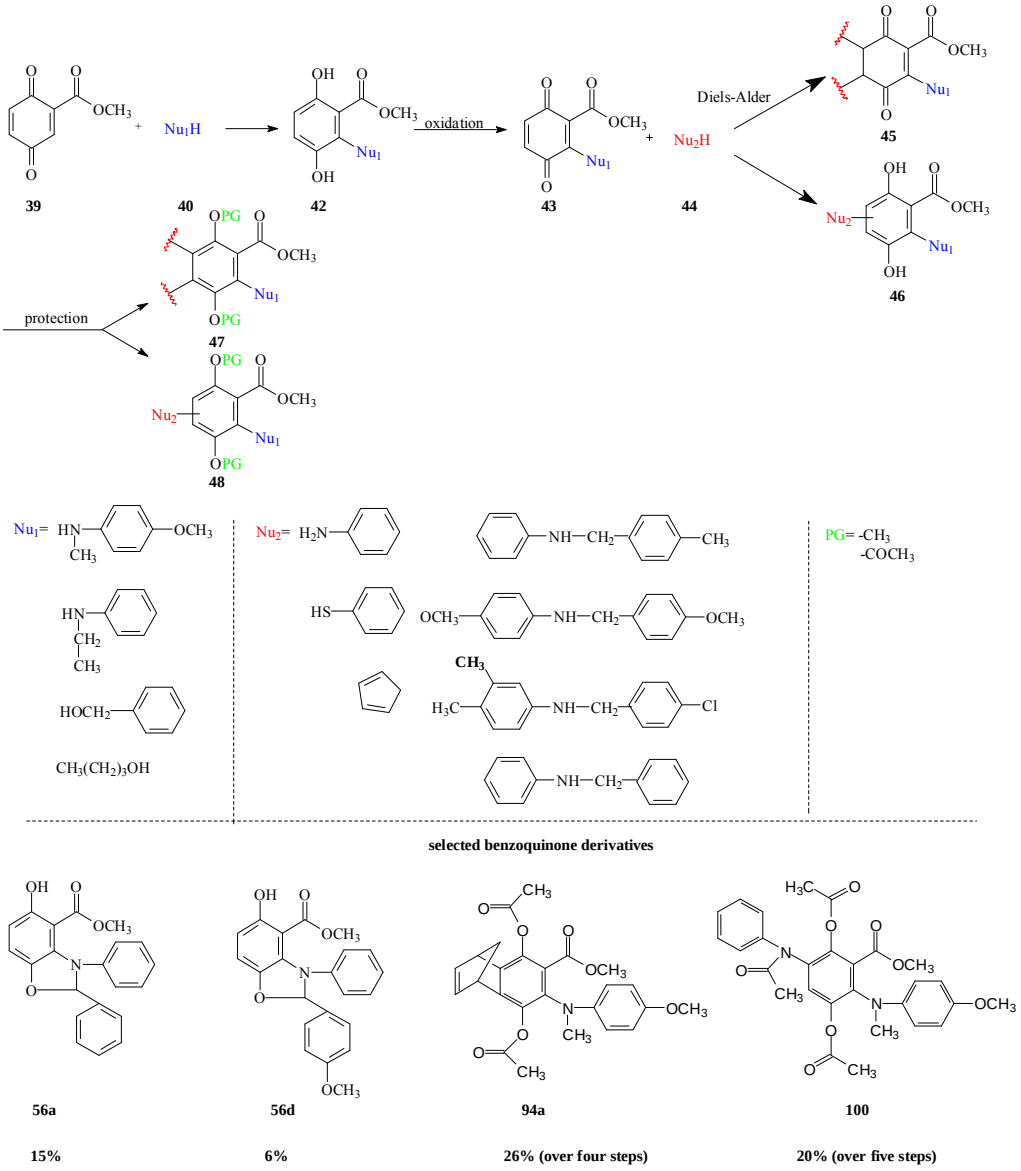


Figure 2: Zusammenfassung der Synthese von 2-Methoxycarbonyl-1,4-benzochinone Derivate in Lösun.

Die biologische Aktivitäten der synthetisierten Benzoquinonederivate wurden anschliessend anhand der verschieden biologischen Assays evaluiert. Die Ergebnisse dieser Untersuchungen sind in der Tabelle 1 zusammengefasst.

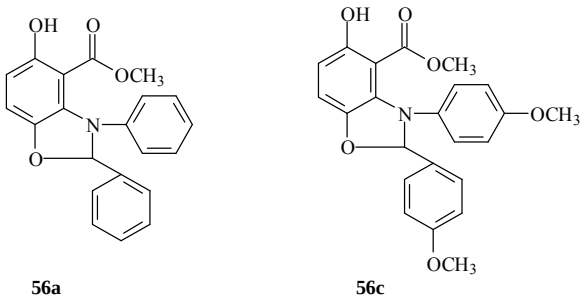
Die ersten biologischen Untersuchungen wurden in Form von cytotoxischen Assays durchgeführt. In diesen zellbasierten Assays zeigten die Benzochinonderivate Cytotoxititätswerte zwischen 7 und 60 µmol.

Tabelle 1. Wachstumsinhibitionsaktivitäten der Verbindungen **96a, 100, 94a, 94b, 96b, 96c, 94c, 56a, 56b, 56c, 56d, 144** mit kultivierten L-929 Mausfibroblasts.

Verbindung	IC50	
	[µg/ml]	[µmol/L]
56a	2.3	7
56c	4.5	11
56d	5.1	14
94a	7	16
94b	15	34
56b	15	36
100	31	60
96a	>400	no inhibition
96b	>400	no inhibition
96c	95	231
94c	54	128
144	53	151

Desweiteren wurden die Wirkunksweise der Benzochinon-Derivate in zahlreichen Immunofluorescence Assays getestet. Sie alle zeigten in Zellkernen interessante phenotypische Effekte, welche in der Stärke und Details voneinander varierten. Es ist aber schwer eine Aussage zu machen, ob diese phenotypischen Effekte aufgrund der underschiedlichen Wirkungsweise der untersuchten Verbindungen auftreten. Die beobachteten Phenomene scheinen sich zu überlappen und bedürfen daher weiterer Untersuchungen.

Anschliessend wurden die Benzochinone Derivate auf Ihre bakterizid, antibiotische und fungal Aktivitäten in mikrobiologischen Assays getestet. Keine der untersuchten Verbindungen zeigten antibakterielle und antifungal Aktivitäten, lediglich zwei der Verbindungen zeigten moderate antibiotische Aktivität (siehe Abb. 3.)



56a **56c**

*Abb. 3: Strukturen der aktiven Verbindungen **56a** and **56c**.*

Der nächster Schritt war die Implementierung des Michael-Addition Konzeptes an der Festphase. Im ersten Schritt der Synthese erfolgt die Addition von Boc-geschützten Nucleophilen als Ester bzw. Ether am Merrifield-Harz, welches dann in einem weiteren Schritt mit 2-Methoxycarbonyl-1,4-benzochinone weiter funktionalisiert wird. Das hiebei entstandene Hydrochinon wird mith DDQ zum Benzochinon oxidiert. Das resultierende

monosubstituierte Benzochinon reagiert in einer weiteren Additionreaktion mit zweitem Nucleophilen unter Bildung vom disubstituerten Intermediat, welches anschliessend durch Alkylierung- bzw. Aceteylierungsreaktionen geschützt wird. Zum Schluss wird das gebildete Produkt von der Festphase unter saurer Konditionen mit $SnCl_4$ abgespalten. Mit dieser Strategie wurden die ersten Syntheseversuche an der Festphase durchgeführt (siehe Abb. 4)

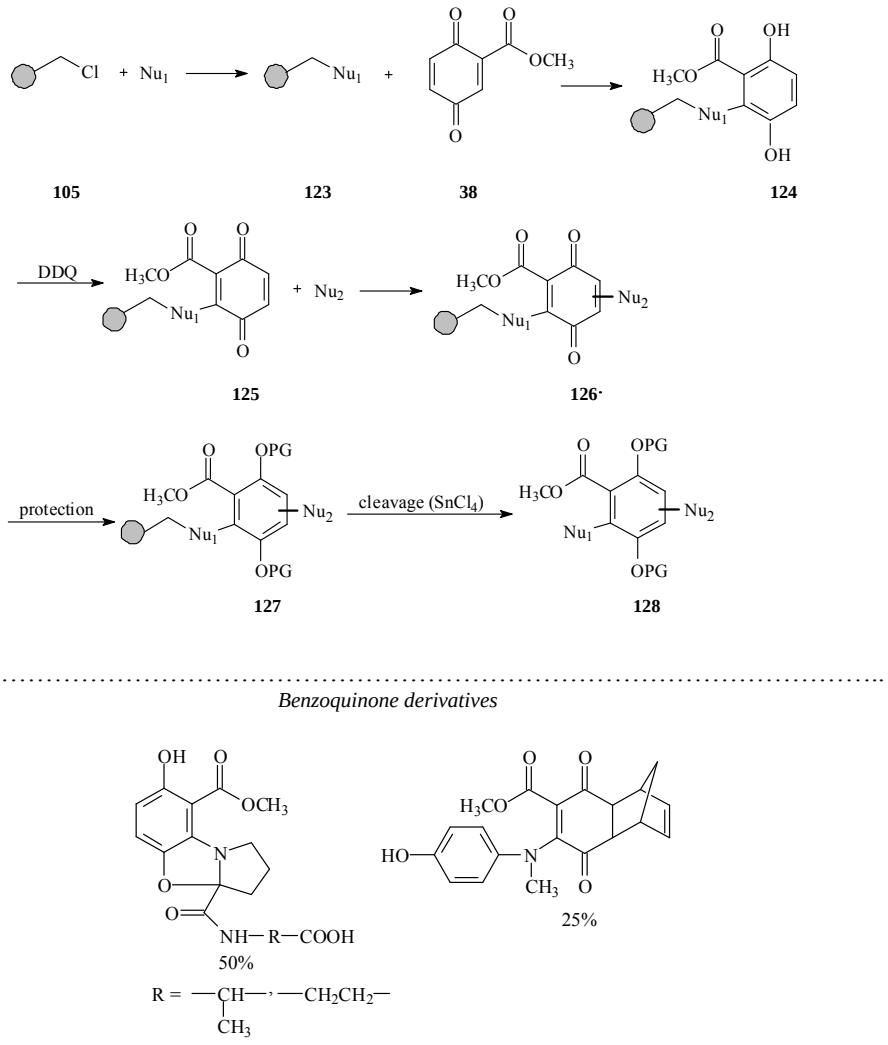
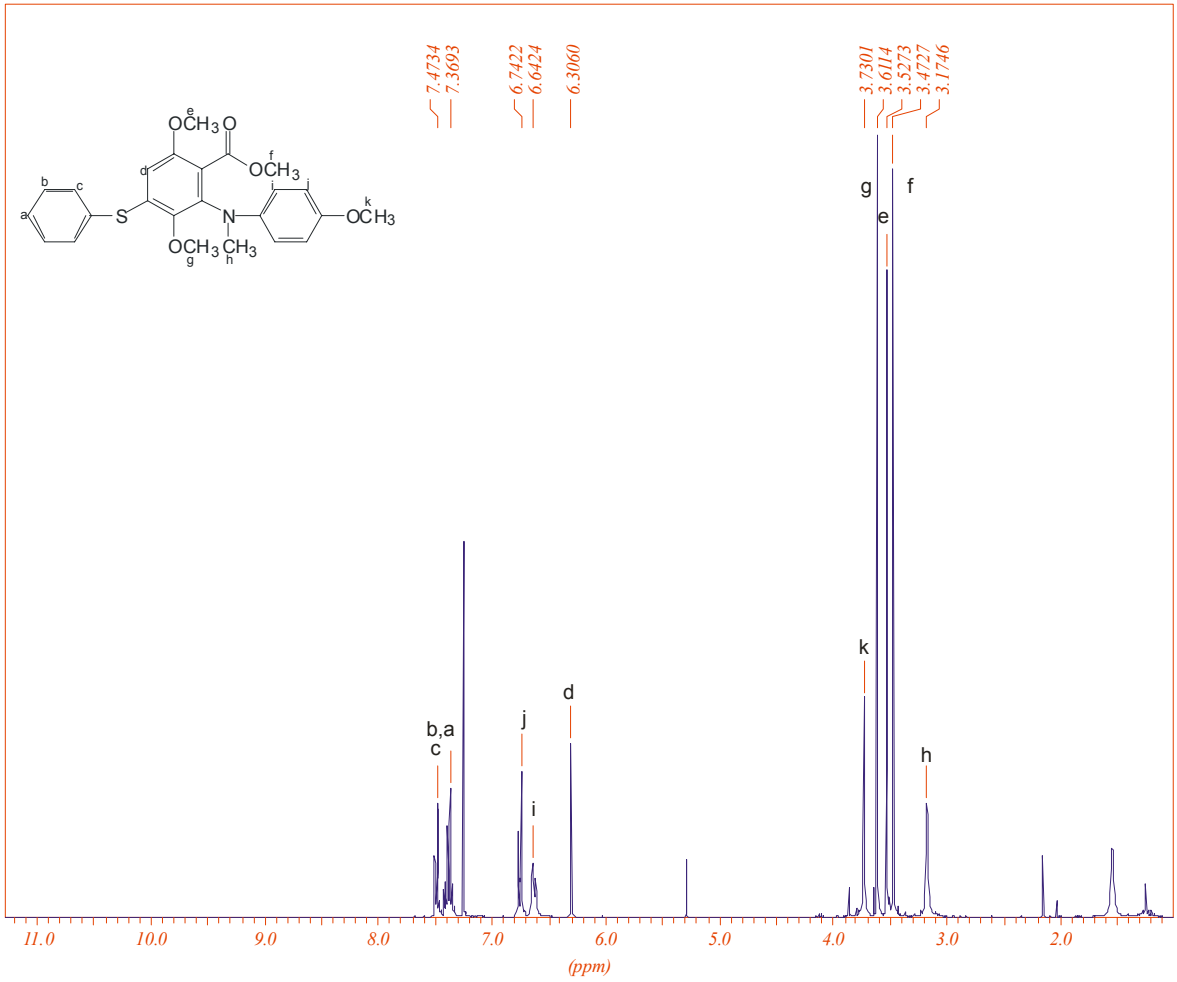


Fig 4:Beispiele für die Synthese von Benzochinonederivate an der Festphase.

Appendix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OCH3
OCH3
OCH3
CH3
S
N
OCH3
a
b
c
d
e
f
g
h
i
j
k
b,a
c
j
i
d
k
g
e
f
h
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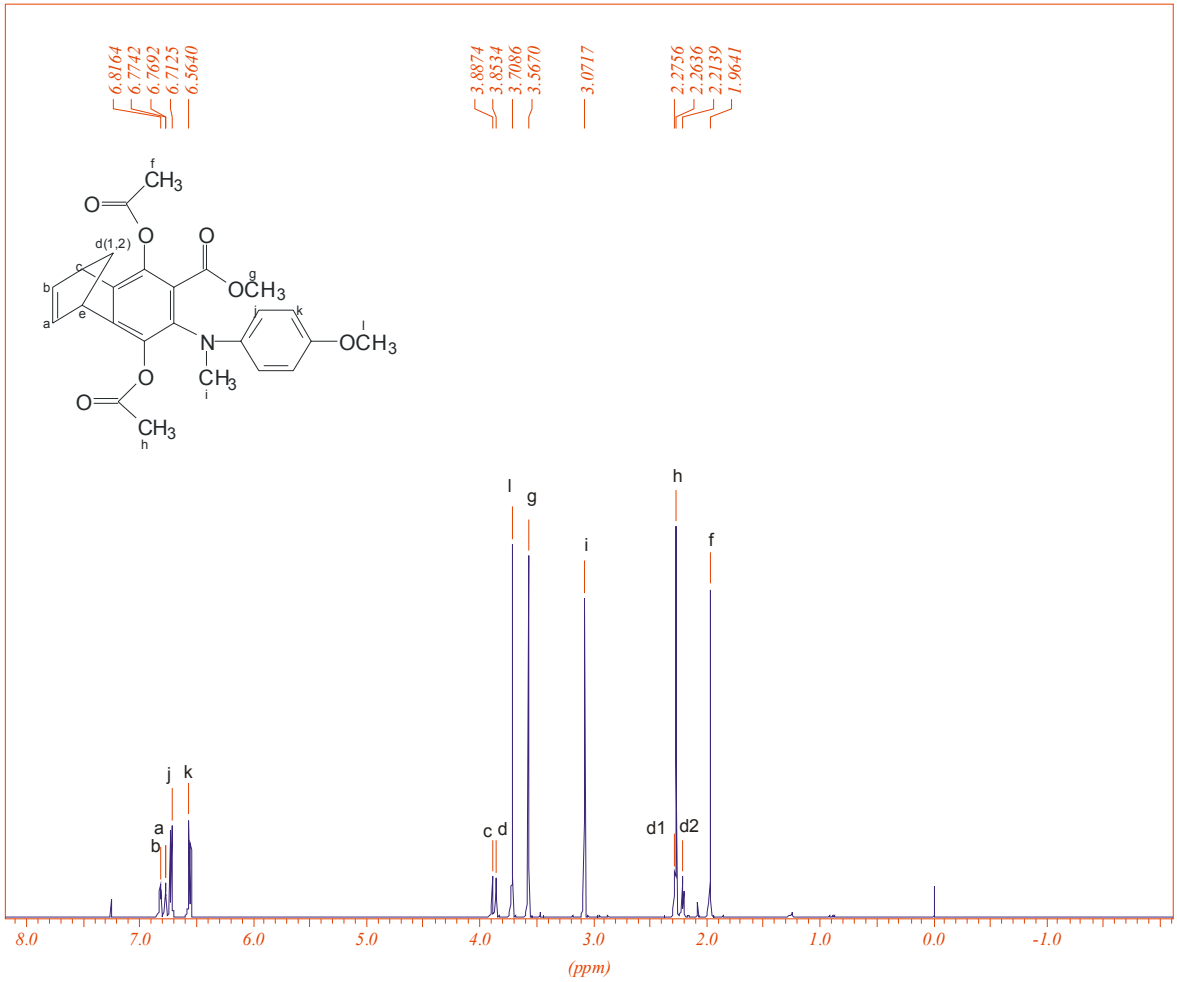
6.0
5.0
4.0
3.0
2.0
(ppm)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2.2756
2.2636
2.2139
1.9641
f
CH3
O
O
d(1,2)
c
b
a
e
O
O
g
OCH3
j
k
l
OCH3
N
CH3
i
O
CH3
h
h
l
g
i
f
j
k
a
b
c
d
d1
d2
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1.0
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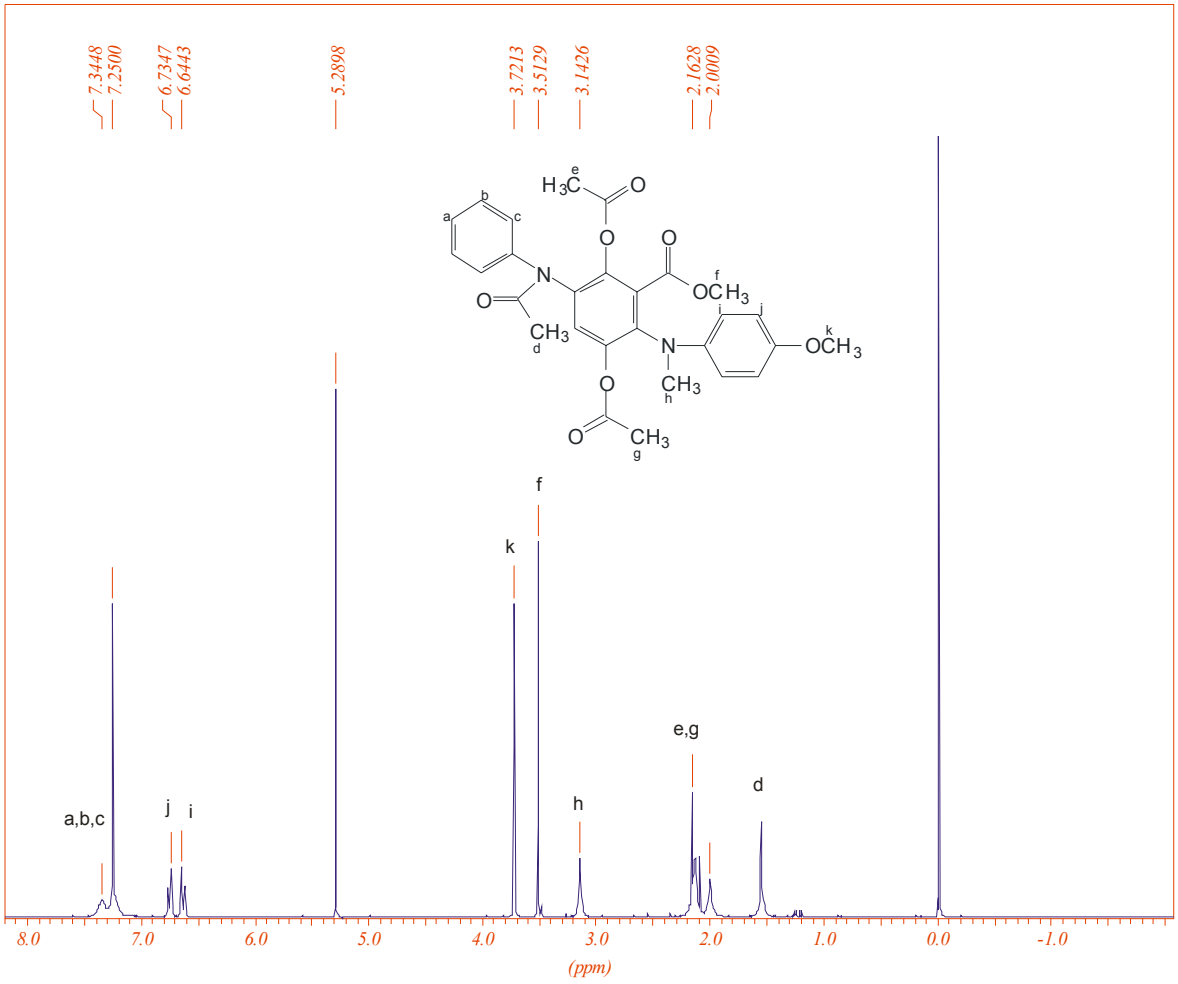
(ppm)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2.1628
2.0009
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j
i
k
f
h
e,g
d
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7.0
6.0
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2.0
1.0
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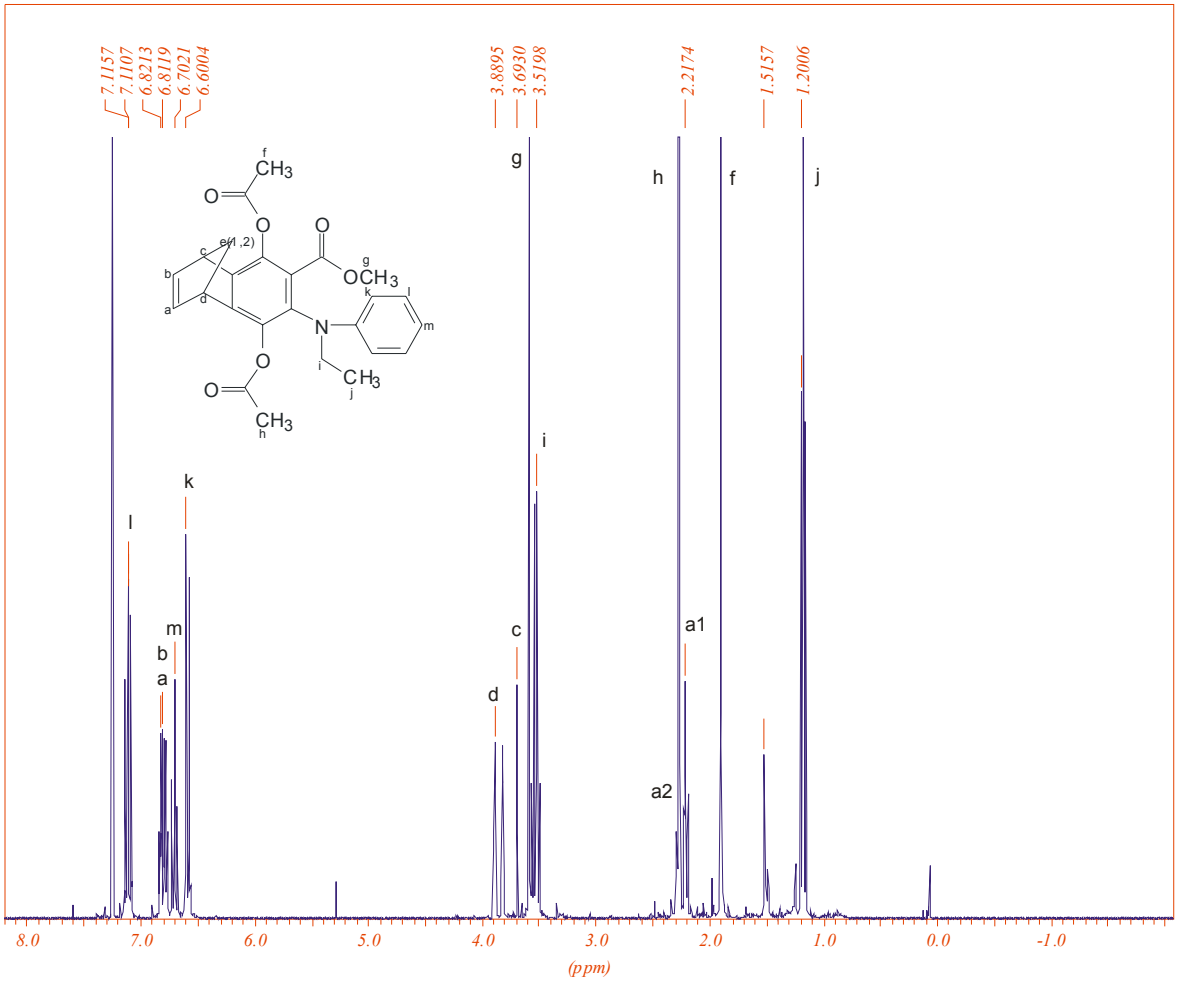
-1.0
(ppm)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1.2006
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CH3
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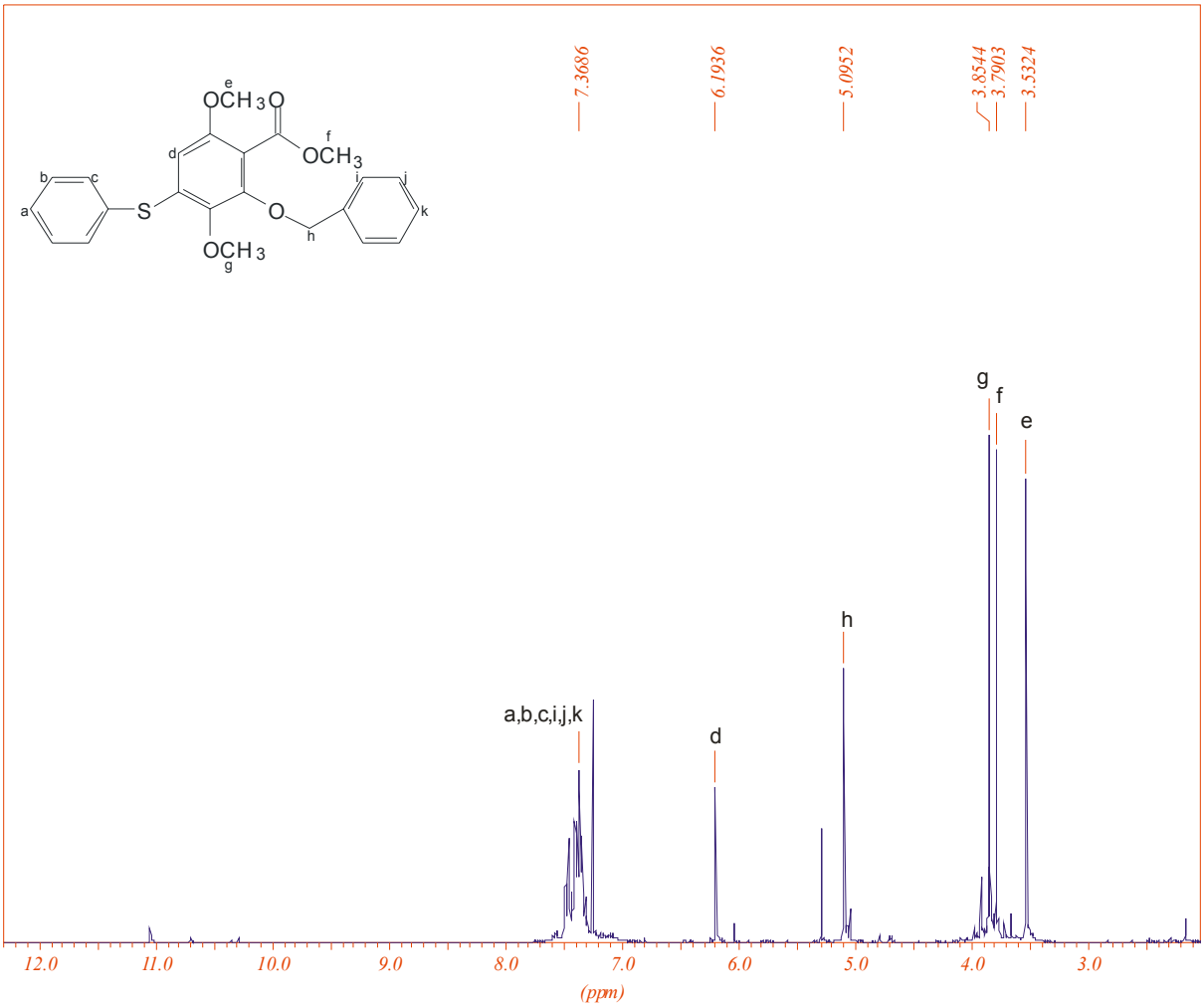
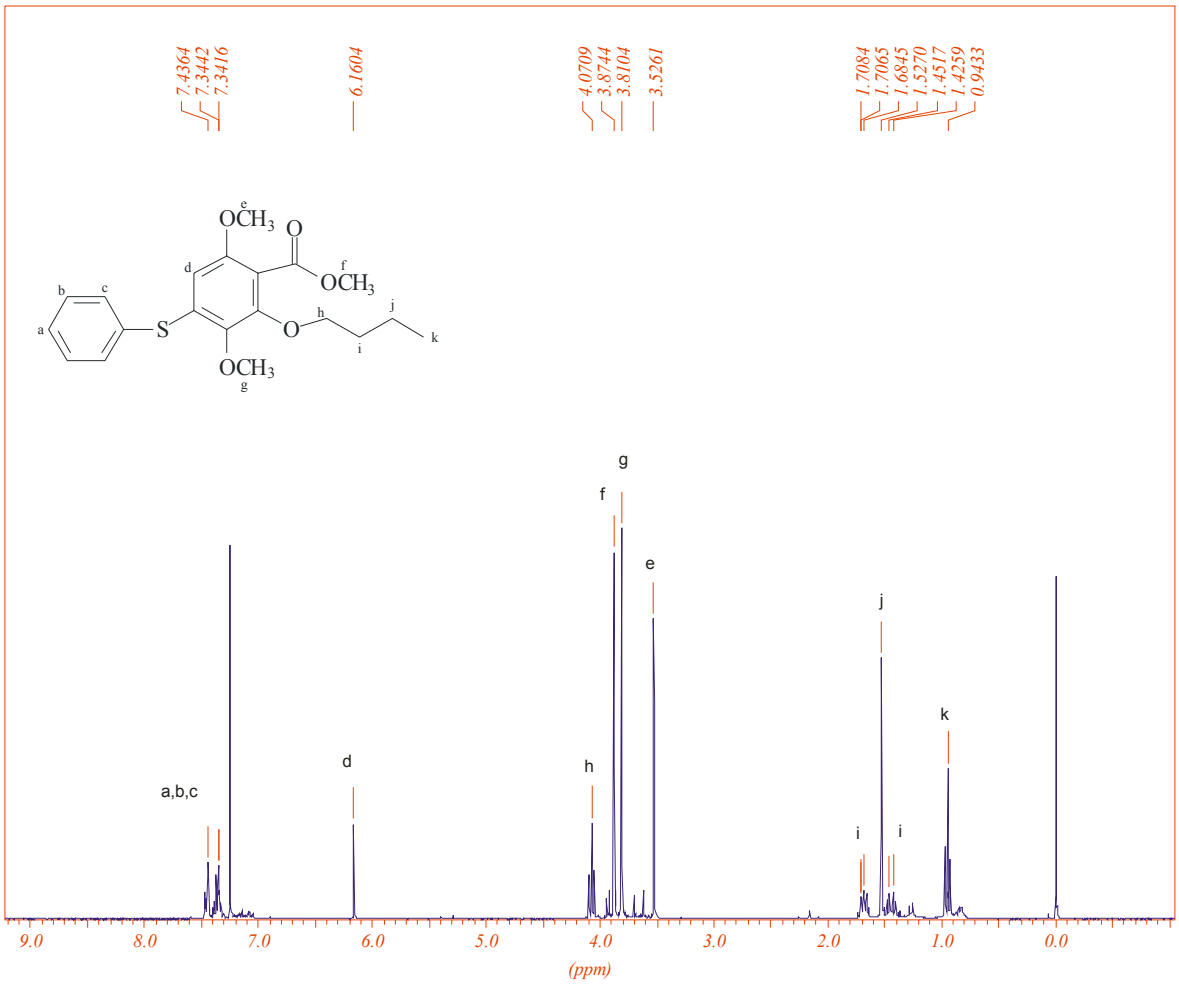
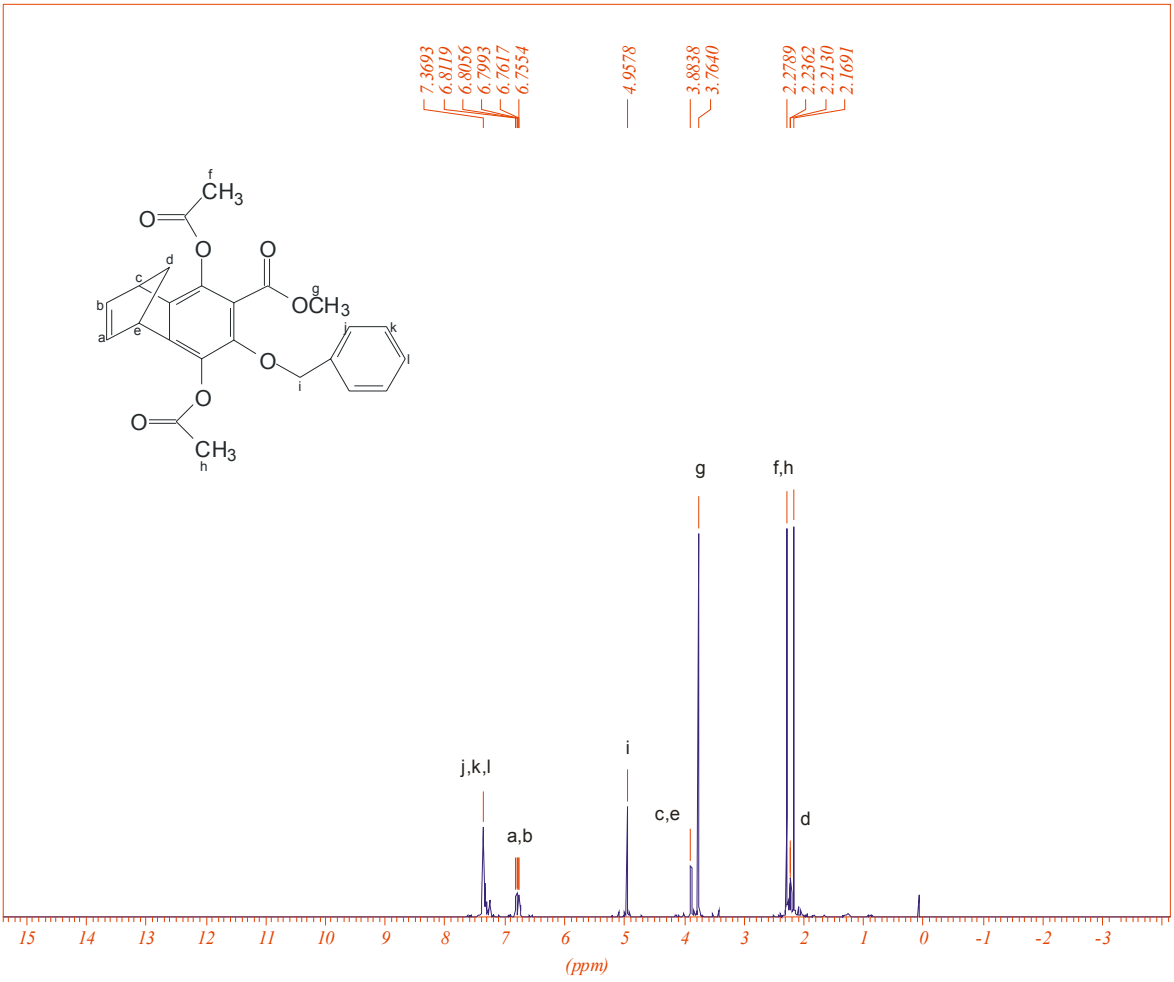
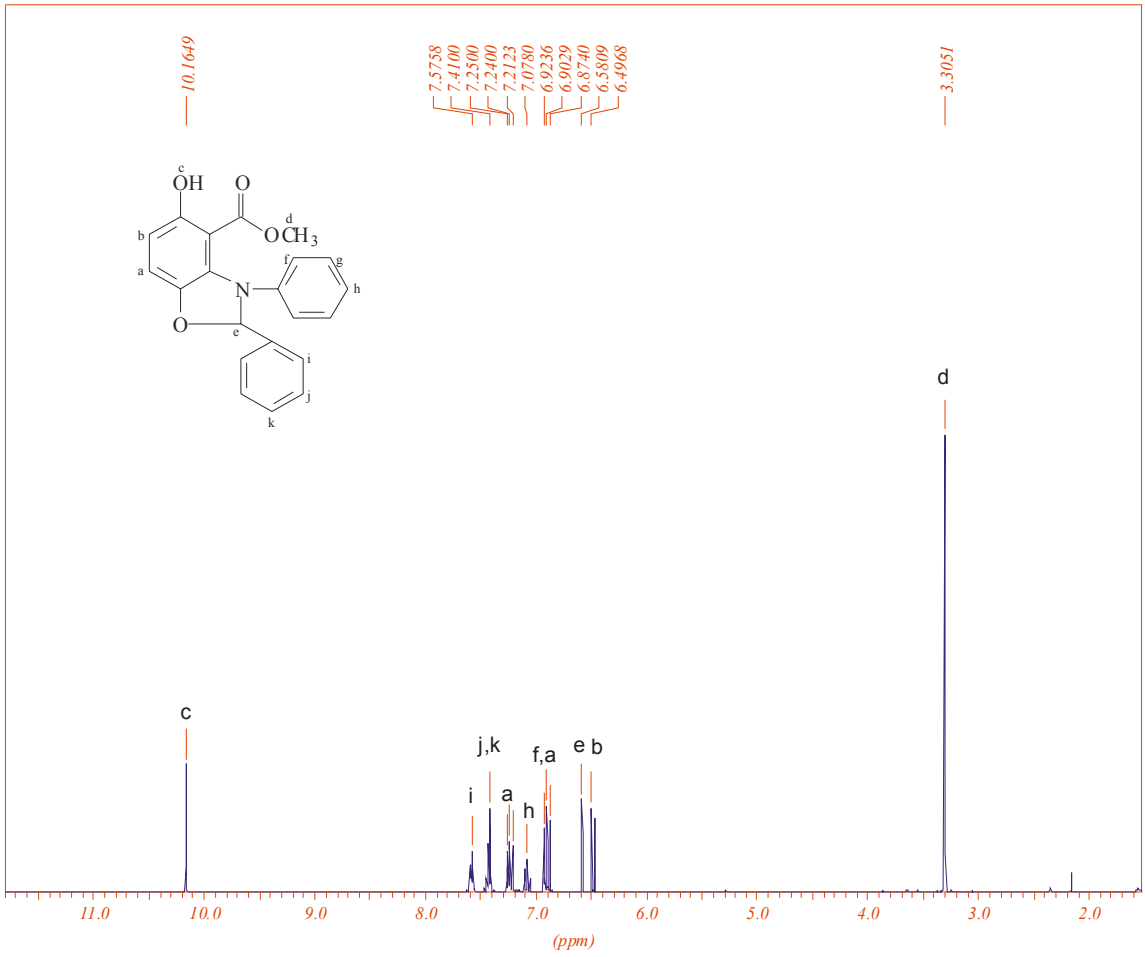
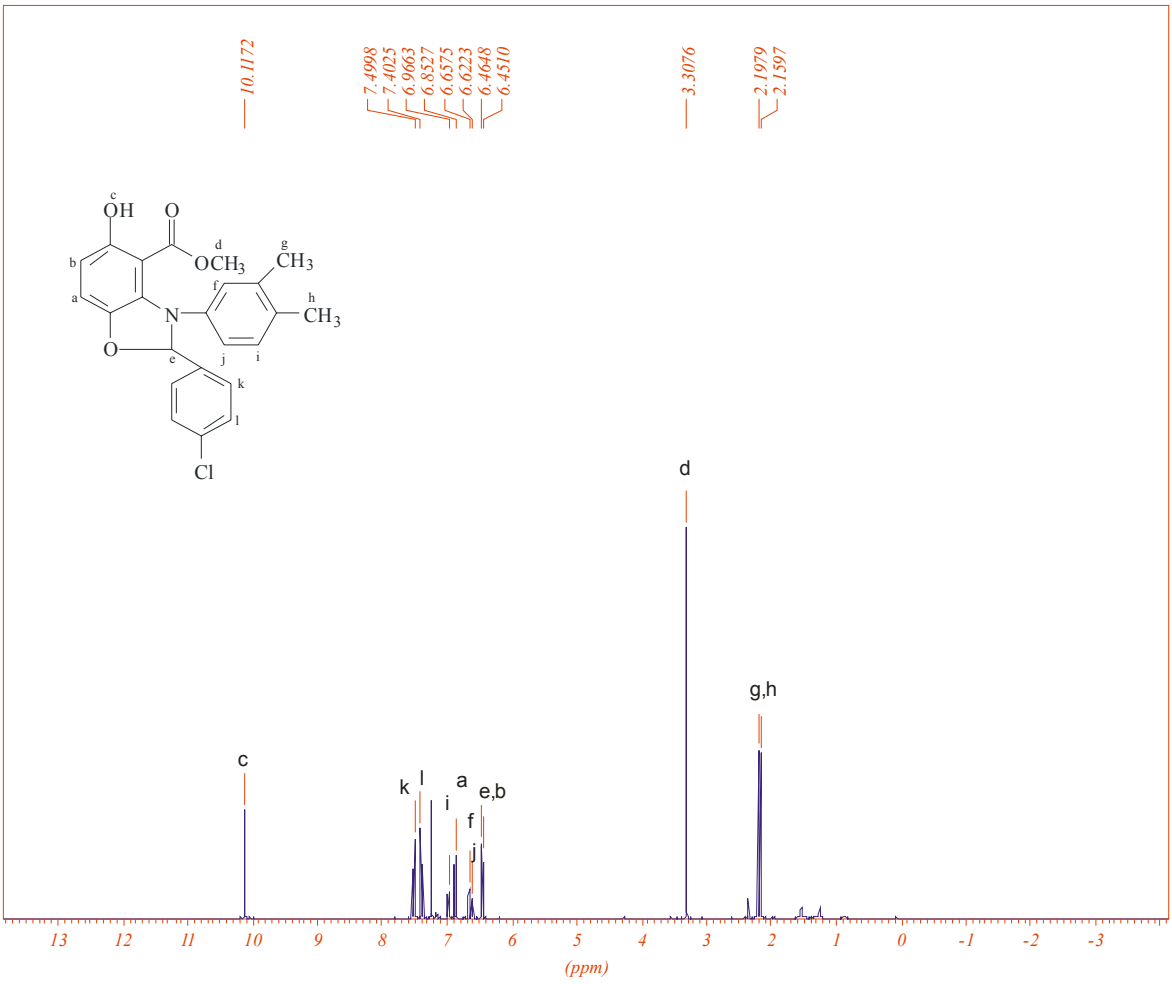
O
O
g
OCH3
k
l
m
N
i
CH3
j
O
O
CH3
h
c
e(1,2)
b
a
d
g
h
f
j
i
k
l
m
b
a
c
d
a1
a2
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
(ppm)


7.3686
6.1936
5.0952
3.8544
3.7903
3.5324
OCH3
OCH3
OCH3
S
O
a
b
c
d
e
f
g
h
i
j
k
a,b,c,i,j,k
d
h
g
f
e
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
(ppm)


7.4364
7.3442
7.3416
6.1604
4.0709
3.8744
3.8104
3.5261
1.7084
1.7065
1.6845
1.5270
1.4517
1.4259
0.9433
OCH3
O
OCH3
S
O
OCH3
a
b
c
d
e
f
g
h
i
j
k
a,b,c
d
h
f
g
e
i
i
j
k
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


7.3693
6.8119
6.8056
6.7993
6.7617
6.7554
4.9578
3.8838
3.7640
2.2789
2.2362
2.2130
2.1691
f
CH3
O
O
O
d
c
b
a
e
g
OCH3
j
k
l
O
i
O
O
CH3
h
g
f,h
i
j,k,l
c,e
a,b
d
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
(ppm)


10.1649
7.5758
7.4100
7.2500
7.2400
7.2123
7.0780
6.9236
6.9029
6.8740
6.5809
6.4968
3.3051
c
OH
O
d
OCH3
b
a
f
g
h
N
O
e
i
j
k
d
c
j,k
f,a
e b
i
a
h
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
(ppm)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7.4998
7.4025
6.9663
6.8527
6.6375
6.6223
6.4648
6.4510
3.3076
2.1979
2.1597
c
OH
O
d
OCH3
g
CH3
b
a
f
h
CH3
N
O
e
j
i
k
l
Cl
d
g,h
c
k
l
i
a
e,b
f
j
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
(ppm)

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